09/604938

PATENT APPLICATION

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

In re United States Patent No. 6,583,272

Inventor: Pascal Sebastian Bailon

Issue Date: June 24, 2003

For: Erythropoietin Conjugates

APPLICATION FOR EXTENSION OF PATENT TERM UNDER 35 U.S.C. §156

Nutley, New Jersey 07110
December 19, 2007

Office of Patent Legal Administration
Room MDW 7D55
600 Dulany Street (Madison Building)
Alexandria, VA 22314

Sir:

Pursuant to 35 U.S.C. § 156, Hoffmann-La Roche Inc. (hereinafter "Roche"), a corporation organized under the laws of the State of New Jersey and owner of U.S. Patent No. 6,583,272 as reflected in an Assignment recorded on December 15, 2000 at reel 011403, frame 0611, submits this Application for extension of the term for the aforementioned patent.

The applicant seeks: (A) an extension of the term of U.S. Patent No. 6,583,272 for 445 days from August 26, 2020 to and including November 14, 2021; and (B) a certification that it is entitled to the rights derived from this patent as set forth in 35 U.S.C. § 156(b).

The information contained in this document and its Exhibits is provided in accordance with 35 U.S.C. § 156(d) and 37 C.F.R. § 1.740 and is listed in the manner set forth in § 1.740.

(1) A Complete Identification of the Approved Product as by Appropriate Chemical and Generic Name, Physical Structure or Characteristics

The approved product, having the trademark MIRCERA®, contains methoxy polyethylene glycol-epoetin beta as the sole active ingredient. Methoxy polyethylene glycol-epoetin beta is formed by reacting a free amino group on an epoetin beta molecule with methoxy polyethylene glycol butanoic acid. The free amino group may be the N-terminal amino group of epoetin beta or the ε-amino group of one of the eight lysine residues of epoetin beta.

Methoxy polyethylene glycol-epoetin beta has the following formula.

$$[CH_3O(CH_2CH_2O)_nCH_2CH_2CH_2CO\text{-}NH]_m — \text{Epoetin beta}$$

with n = ~681 and m = 1

MIRCERA® has been approved as a sterile, preservative-free protein solution for intravenous or subcutaneous administration. Single use vials have been approved containing 50, 100, 200, 300, 400, 600, or 1000 mcg in 1 mL solution of MIRCERA®. Single use pre-filled syringes have been approved containing 50, 75, 100, 150, 200, or 250 mcg in 0.3 mL solution of MIRCERA® and 400, 600, or 800 mcg in 0.6 mL solution of MIRCERA®. In addition to methoxy polyethylene glycol-epoetin beta, the solution also contains sodium phosphate, sodium sulfate, mannitol, methionine, and poloxamer 188.

A copy of the approved product label is annexed as Exhibit A.

The approved therapy for the approved product is the treatment of anemia associated with chronic renal failure.

The term "approved product" is defined in 35 U.S.C. § 156(a) as the "product" referred to in paragraphs (4) and (5) of subsection (a). In turn, the word "product" is defined in 35 U.S.C. § 156(f)(1)(A) to comprise a "drug product" which is described in 35 U.S.C. § 156(f)(2) to include "the active ingredient of a new drug, antibiotic drug, or human biological product . . . including any salt or ester of the active ingredient, as a single entity or in combination with another active ingredient." The approved product subject to this Application, MIRCERA®, thus includes methoxy polyethylene glycol-epoetin beta and any salts and esters thereof, as its active ingredient, as a single entity or in combination with another active ingredient.

(2) A Complete Identification of the Federal Statute Including the Applicable Provision of Law under which the Regulatory Review Occurred

The regulatory review occurred under Section 351 of the Public Health Service Act (Title 42, United States Code).

(3) An Identification of the Date on which the Product Received Permission for Commercial Marketing or Use under the Provision of Law under which the Applicable Regulatory Review Period Occurred

MIRCERA® was approved by the Food and Drug Administration ("FDA") for commercial marketing or use under Section 351 of the Public Health Service Act on November 14, 2007. A copy of the FDA approval letter is annexed hereto as Exhibit B.

(4) In the Case of a Drug Product, an Identification of Each Active Ingredient in the Product and, as to Each Active Ingredient, a Statement that It has not been Previously Approved for Commercial Marketing or Use under the Federal Food, Drug, and Cosmetic Act, the Public Health Service Act, or the Virus-Serum-Toxin Act, or a Statement of when the Active Ingredient was Approved for Commercial Marketing or Use (Either Alone or in Combination with Other Active Ingredients), the Use for which It was Approved, and the Provision of Law under which it was Approved

The sole active ingredient in the approved product is methoxy polyethylene glycol-epoetin beta which active ingredient has not been previously approved for commercial marketing or use under the Food, Drug, and Cosmetic Act, The Public Heath Service Act, or the Virus-Serum-Toxin Act.

(5) A Statement that the Application is Being Submitted within the Sixty Day Period Permitted for Submission Pursuant to 37 C.F.R. § 1.720(f) and an Identification of the Date of the Last Day on which the Application Could be Submitted

This application is being submitted within the permitted sixty (60) day period, the last day of which is January 13, 2008.

(6) A Complete Identification of the Patent for which an Extension is Being Sought by the Name of the Inventor, the Patent Number, the Date of Issue, and the Date of Expiration

The complete identification of the patent for which an extension is being sought is:

Inventor: Pascal Sebastian Bailon

Patent No: 6,583,272

U.S. Patent No. 6,583,272
Issue Date: June 24, 2003

Issue Date: June 24, 2003

Expiration Date: August 26, 2020 (without extension)

(7) A Copy of the Patent for which an Extension is Being Sought, Including the Entire Specification (Including Claims) and Drawings

A copy of U.S. Patent No. 6,583,272 is annexed as Exhibit C.

(8) A Copy of Any Disclaimer, Certificate of Correction, Receipt of Maintenance Fee Payment, or Reexamination Certificate Issued In the Patent

No Disclaimer or Reexamination Certificate has been issued for U.S. Patent No. 6,583,272. A copy of the maintenance fee payment receipt for the 4th year payment from the record of the U.S. Patent and Trademark Office through its web-site for U.S. Patent No. 6,583,272 is annexed as Exhibit D. A Certificate of Correction was issued on the patent on May 23, 2007 and a copy thereof is attached to the aforementioned copy of the patent in Exhibit C.

(9) A Statement that the Patent Claims the Approved Product, or a Method of Using or Manufacturing the Approved Product, and a Showing which Lists Each Applicable Patent Claim and Demonstrates the Manner in which at Least One Such Patent Claim Reads on: (i) The Approved Product, if the Listed Claims Include any Claim to the Approved Product; (ii) The Method of Using the Approved Product, if the Listed Claims Include any Claim to the Method of Using the Approved Product; and (iii) The Method of Manufacturing the Approved Product, if the Listed Claims Include any Claim to the Method of Manufacturing the Approved Product

United States Patent No. 6,583,272 claims the approved product in product claims 1 to 3 and 5 to 9.

U.S. Patent No. 6,583,272
Issue Date: June 24, 2003

Claim 1 reads as follows.

1. A conjugate comprising an erythropoietin glycoprotein having a free amino group and having the in vivo biological activity of causing bone marrow cells to increase production of reticulocytes and red blood cells and selected from the group consisting of human erythropoietin and analogs thereof which analogs have sequence of human erythropoietin modified by the addition of from 1 to 6 glycosylation sites or a rearrangement of at least one glycosylation site; said glycoprotein being covalently linked to a poly(ethylene glycol) group of the formula $-CO-(CH_2)_x-(OCH_2CH_2)_m-OR$ by the -CO of said poly(ethylene glycol) group forming an amide bond with said free amino group; wherein R is lower alkyl; x is 2 or 3; m is from about 450 to about 900; and m is chosen so that the molecular weight of the conjugates minus the erythropoietin glycoprotein is from 20 kilodaltons to 100 kilodaltons.

The approved product is a molecule as defined in claim 1 in which the glycoprotein is human epoetin beta (a human erythropoietin) and the "poly(ethylene glycol) group" (as recited in the claim) is one of the formula therein in which x is 3, m is approximately 681, and R is methyl. The molecular weight of the conjugate minus the glycoprotein is about 30 kDa (see Exhibit A, page 12, section 11).

Claim 2 reads as follows.

2. The conjugate of claim 1 of the formula:

$$P-NHCO-(CH_2)_x-(OCH_2CH_2)_m-OR \qquad (I)$$

wherein m, x and R are as above and P is the residue of the glycoprotein without the free amino group which forms the amide linkage.

The approved product is a molecule as defined in claim 2 in which x is 3, m is approximately 681, R is methyl, and P is the "residue" of a glycoprotein, the glycoprotein being human epoetin beta (a human erythropoietin), without the free amino group which forms the amide linkage in formula I above. "Residue" as it relates to the phrase "residue of the glycoprotein" is defined in the specification as being the glycoprotein without the amino group(s) that forms an amide linkage with the carbonyl group in formula (I) above (see column 4, lines 1 to 4 of the `272 patent).

Claim 3 reads as follows.

> 3. The conjugate of claim 2 wherein the glycoprotein is human erythropoietin.

The approved product is a conjugate as defined in claim 2 in which the glycoprotein that is used in the making thereof is human epoetin beta (a human erythropoietin).

Claim 5 reads as follows.

> 5. The conjugate of claim 3, wherein the glycoprotein has the sequence SEQ ID NO: 1.

The approved product is a conjugate as defined in claim 3 in which the glycoprotein used in the making thereof has the sequence of SEQ ID NO: 1.

Claim 6 reads as follows.

> 6. The conjugate of claim 5, wherein R is methyl.

The approved product is a conjugate as defined in claim 5 in which R is methyl.

Claim 7 read as follows.

7. The conjugate of claim 5 wherein x is 3.

The approved product is a conjugate as defined in claim 5 in which x is 3.

Claim 8, as corrected by the aforementioned Certificate of Correction, reads as follows.

8. The conjugate of claim 7 wherein said molecular weight is from about 20 kDa to about 40 kDa.

The approved product is a conjugate as defined in claim 7 in which $-CO-(CH_2)_x-(OCH_2CH_2)_m-OR$ has a molecular weight of about 30 kDa (see Exhibit A, page 12, section 11).

Claim 9 reads as follows.

9. The conjugate of claim 8 wherein said molecular weight is about 30 kDa.

The approved product is a conjugate as defined in claim 8 in which $-CO-(CH_2)_x-(OCH_2CH_2)_m-OR$ has a molecular weight of about 30 kDa (see Exhibit A, page 12, section 11).

In view of the above, the aforementioned claims read on the approved product.

(10) A Statement, Beginning on a New Page, of the Relevant Dates and Information Pursuant to 35 U.S.C. § 156(g) in Order to Enable the Secretary of Health and Human Services or the Secretary of Agriculture, as Appropriate, to Determine the Applicable Regulatory Review Period as Follows: (i) For a Patent Claiming a Human Drug Product, Antibiotic, or Human Biological Product, The Effective Date of the Investigational New Drug (IND) Application and the IND Number; the Date on Which a New Drug Application (NDA) or a Product License Application (PLA) was Initially Submitted and the NDA or PLA Number and the Date on which the NDA was Approved or the Product License Issued

a)	Effective date of the investigational new drug application (IND) and IND number:	January 3, 2002 (Exhibit E-1: IND Cover Letter; Exhibit E-2: FDA acknowledgement)
	IND number:	BB IND 10158
b)	Date on which a Biologics License Application (BLA) was initially submitted and BLA number:	April 18, 2006 (Exhibit F-1: BLA Cover Letter; Exhibit F-2: FDA acknowledgement)
	BLA number:	BL 125164/0
c)	Date on which BLA was approved:	November 14, 2007 (Exhibit B)

(11) A Brief Description Beginning on a New Page of the Significant Activities Undertaken by the Marketing Applicant during the Applicable Regulatory Review Period with Respect to the Approved Product and the Significant Dates Applicable to Such Activities

A chronology of significant activities undertaken by the applicant during the regulatory review period with respect to the approved product is annexed as Exhibit G. This Exhibit specifically is directed to the communications between the applicant and the FDA. The Exhibit provides the nature of each correspondence with the FDA, including a brief summary of its subject matter, and the date of the correspondence. For convenience, the chronology is divided into two sections: "Correspondence Log - Testing Phase" and "Correspondence Log - Application Phase."

If necessary, the applicant reserves the right to supplement its chronology in Exhibit G with materials from which it was derived and other evidence related to the applicant's activities in obtaining the approval of MIRCERA®. *See, e.g.,* 21 C.F.R. § 60.32.

(12) A Statement Beginning on a New Page That in the Opinion of the Applicant the Patent is Eligible for the Extension and a Statement as to The Length of the Extension Claimed, Including How the Length of Extension was Determined

Eligibility

Under the law and in the opinion of the applicant, U.S. Patent No. 6,583,272 is eligible for an extension under 35 U.S.C. § 156.

In particular, 35 U.S.C. § 156(a) in its relevant parts, provides that the term of a patent shall be extended if the following requirements are satisfied: (1) the patent claims a product, a method of using a product or a method of manufacturing a product; (2) the term of the patent has not expired before an application for extension is submitted; (3) the term of the patent has never been extended; (4) an application for extension is submitted by the owner of record of the patent or its agent and in accordance with 35 U.S.C. § 156(d); (5) the product has been subject to a regulatory review period as defined in 35 U.S.C. § 156(a) before its commercial marketing or use; and (6) the permission for the commercial marketing or use of the product after the regulatory review period is the first permitted commercial marketing or use of the product under the provision of law under which such regulatory review period occurred.

These requirements are met as follows:

1. U.S. Patent No. 6,583,272 claims a product.

2. The term of U.S. Patent No. 6,583,272 presently will expire on August 26, 2020 and thus, the patent has not expired before submission of this Application.

3. The term of U.S. Patent No. 6,583,272 has never been extended under 35 U.S.C. § 156.

4. This Application is submitted by Roche, the owner of record of U.S. Patent No. 6,583,272. This Application is submitted in accordance with 35 U.S.C. § 156(d) and 37 C.F.R. § 1.740 within the sixty (60) day period beginning on November 14, 2007 and ending on January 13, 2008. The product received permission for marketing or use under the Public Health Service Act. This Application contains the information required under 35 U.S.C. § 156(d) and 37 C.F.R. § 1.740.

5. The product was subject to a regulatory review period under Section 351 of the Public Health Service Act before its commercial marketing or use, as evidenced by the chronology (Exhibit G) and the Letter of Approval from the FDA, dated November 14, 2007 (Exhibit B).

6. The permission for the commercial marketing or use of the approved product after the regulatory review period is the first permitted commercial marketing or use of a product having methoxy polyethylene glycol-epoetin beta in any form as its active ingredient, under the provisions of the Public Health Service Act under which such regulatory review period occurred. This is confirmed by the absence of any approved drug application for the active ingredient (methoxy polyethylene glycol-epoetin beta) of the approved product (MIRCERA®) in any form prior to November 14, 2007.

Accordingly, U.S. Patent No. 6,583,272 satisfies the requirements for an extension under 35 U.S.C. § 156.

U.S. Patent No. 6,583,272
Issue Date: June 24, 2003

Length

In the opinion of the applicant, the term of U.S. patent No. 6,583,272 should be extended for 445 days from August 26, 2020 to and including November 14, 2021.

This extension was determined on the following basis:

Testing Phase (37 C.F.R. § 1.775(c) (1))

For the approved product, that portion of the regulatory review period as defined in 35 U.S.C. 156 (g) (1) (B) (i) ("Testing Phase") commenced on January 3, 2002 and ended on April 18, 2006, which is 1566 days.

Application Phase (37 C.F.R. § 1.775(c) (2))

For the approved product, that portion of the regulatory review period as defined under 35 U.S.C. 156 (g) (1) (B) (ii) ("Application Phase") commenced on April 18, 2006 and ended on November 14, 2007 which is 575 days.

Regulatory Review Period (37 C.F.R. § 1.775(c))

As defined in 35 U.S.C. 156 (g) (1) (B), the regulatory review period is the sum of the Testing Phase and the Application Phase, which is a total of 2141 days.

Reduction for Review Prior to the Issue of The Patent (37 C.F.R. § 1.775(d)(1)(i))

The applicable regulatory review period is reduced by that period of review occurring before and on the date the patent issued.

U.S. Patent No. 6,583,272
Issue Date: June 24, 2003

U.S. Patent No. 6,583,272 (Exhibit C) issued June 24, 2003 and the effective date of the IND was January 3, 2002. Accordingly, a reduction of 537 days, all occurring in the Testing Phase, is applicable for the review period prior to the issue of the patent, leaving a revised Testing Phase of 1029 days and a revised regulatory review period of 1604 days.

Due Diligence Reduction to Regulatory Review Period (37 C.F.R. § 1.775(d)(1)(ii))

Under 35 U.S.C. § 156(c)(1), the Testing Phase and Application Phase of the regulatory review period are reduced by the period during which the applicant for the patent extension, in the regulatory review period, did not act with due diligence. In the opinion of the applicant and as illustrated by the chronology in Exhibit G, the applicant acted with due diligence during both periods of time. Thus, there is no reduction in the regulatory review period because of lack of due diligence.

One-Half Testing Phase Reduction (37 C.F.R. § 1.775(d)(1)(iii))

Under 35 U.S.C. § 156(c)(2), the regulatory review period is reduced by one-half of the remaining 1029 day Testing Phase. This is 514 days. Thus, the remaining 1604 day regulatory review period is further reduced by 514 days, leaving a final revised regulatory review period of 1090 days.

Fourteen Year Cap (37 C.F.R. § 1.775(d)(2) - (4))

Under 35 U.S.C. § 156(c)(3), should the period of time remaining in the term of the patent after the date of approval when added to the period of extension exceed fourteen (14) years, the period of extension is reduced so that the total of both such periods does not exceed fourteen (14) years. In applying section 156(c)(3), the final revised regulatory review period as calculated above

(1090 days) is added onto the end of the original term of the patent, August 26, 2020, resulting in a date of August 21, 2023. Alternatively, fourteen (14) years is added to the BLA approval date (November 14, 2007) resulting in a date of November 14, 2021. The earlier of the above two dates, November 14, 2021, is thus selected.

Two and Five Year Extension Limits (37 C.F.R. § 1.775(d)(5) & (6))

A patent issued after September 24, 1984 is limited to a maximum extension of five years.

U.S. Patent No. 6,583,272 (Exhibit C) issued on June 24, 2003. Accordingly, the patent is eligible for an extension of up to five years.

As set forth above, the term of U.S. Patent No. 6,583,272 is eligible for an extension of 445 days to November 14, 2021.

(13) A Statement That Applicant Acknowledges a Duty to Disclose to the Commissioner of Patents and Trademarks and the Secretary of Health and Human Services any Information Which is Material to the Determination of Entitlement to the Extension Sought

The applicant acknowledges a duty to disclose to the Commissioner of Patents and Trademarks ("the Commissioner") and the Secretary of Health and Human Services ("the Secretary") any information which is material to any determinations of entitlement to the extension sought in the Application.

(14) The Prescribed Fee for Receiving and Acting Upon the Application for Extension

The applicant encloses (in duplicate) a transmittal letter requesting the amount of ***$1120.00*** be charged to Account No. 08-2525.

(15) The Name, Address and Telephone Number of the Person to Whom Inquiries and Correspondence Relating to the Application for Patent Term Extension are to be Directed

Please address all correspondence to:

George W. Johnston
Hoffmann-La Roche Inc.
Patent Law Department
340 Kingsland Street
Nutley, New Jersey 07110

Please direct all telephone calls to:

Gene J. Yao
(973) 235-6993

(16) Two Additional Copies of These Application Papers

Two additional copies of these application papers are enclosed pursuant to paragraph (b) of 37 C.F.R. § 1.740.

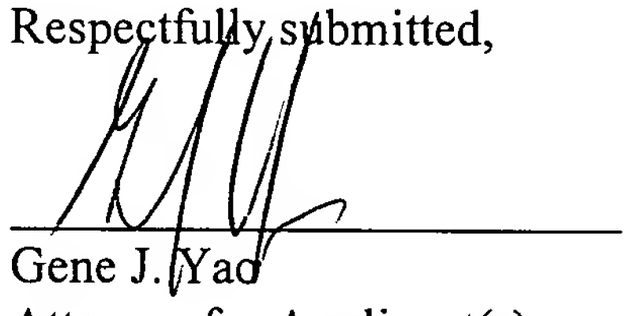
(17) An Oath or Declaration

The applicant attaches a declaration signed by an officer of Roche, the owner of record of U.S. Patent No. 6,583,272, who is authorized to practice before the Patent and Trademark Office and who has general authority to act on Roche's behalf in patent matters.

Request for Extension

Having included in this Application all of the requisite information under 35 U.S.C. § 156 and 37 C.F.R. § 1.740, the applicant requests (i) an extension of U.S. Patent No. 6,583,272 for 445 days from August 26, 2020 to and including November 14, 2021, by reason of its claims encompassing the approved product and (ii) certification that it is entitled to the rights derived from this patent as set forth in 35 U.S.C. § 156(b).

Respectfully submitted,



Gene J. Yao
Attorney for Applicant(s)
(Reg. No. 47,193)
340 Kingsland Street
Nutley, New Jersey 07110
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PATENT APPLICATION

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

In re United States Patent No. 6,583,272

Inventor: Pascal Sebastian Bailon

Issue Date: June 24, 2003

For: Erythropoietin Conjugates

DECLARATION AND POWER OF ATTORNEY FOR APPLICATION FOR EXTENSION OF PATENT TERM UNDER 35 U.S.C. §156

Nutley, New Jersey 07110
December 17, 2007

Office of Patent Legal Administration
Room MDW 7D55
600 Dulany Street (Madison Building)
Alexandria, VA 22314

Sir:

I, George W. Johnston, a Vice President of Hoffmann-La Roche Inc. ("ROCHE"), which submits the attached Application for Extension of Patent Term Under 35 U.S.C. § 156, of the same date as this Declaration, declare that:

(1) ROCHE is the owner of U.S. Patent No. 6,583,272;

(2) I am a patent attorney authorized to practice before the Patent and Trademark Office and have authority from ROCHE to act on its behalf in patent matters;

(3) I have reviewed and understand the contents of the Application being submitted for extension of the term of U.S. Patent No. 6,583,272 pursuant to 35 U.S.C. § 156 and 37 C.F.R. § 1.710 et seq;

(4) I believe this patent is subject to extension under 35 U.S.C. § 156 and 37 C.F.R. § 1.710;

(5) I believe an extension of the length claimed is justified under 35 U.S.C. § 156 and the applicable regulations; and

(6) I believe the patent for which the extension is being sought meets the conditions for extension of the term of a patent as set forth in 35 U.S.C. § 156, and more particularly, in 37 C.F.R. § 1.720.

I hereby appoint the following attorneys as agents under 35 U.S.C. § 156 with the authority to sign, submit and prosecute this Application and transact all business in the Patent and Trademark Office and with the Secretary of Health and Human Services connected therewith: George W. Johnston (Reg. No. 28090), Patricia S. Rocha-Tramaloni (Reg. No. 31054) and Gene J. Yao (Reg. No. 47193).

Send correspondence to:

George W. Johnston
Hoffmann-La Roche Inc.
Patent Law Department
340 Kingsland Street
Nutley, New Jersey 07110

Direct telephone calls to:

Gene J. Yao
(973) 235-6993

I declare further that all statements made herein of my own knowledge are true and that all statements made on information and belief are believed to be true; and further that these statements were made with the knowledge that willful false statements and the like so made are punishable by fine or imprisonment, or both, under Section 1001 of Title 18 of the United States

Code and that such willful false statements may jeopardize the validity of this patent extension application or any extension of U.S. Patent No. 6,583,272.

Respectfully submitted,
HOFFMANN-LA ROCHE INC.

By: _____
George W. Johnston
Vice President

Date: Dec 17, 2007

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HIGHLIGHTS OF PRESCRIBING INFORMATION

These highlights do not include all the information needed to use Mircera safely and effectively. See full prescribing information for Mircera.

Mircera® (methoxy polyethylene glycol-epoetin beta)

Solution for Injection: Intravenous [IV] or Subcutaneous [SC] use
Initial U.S. Approval: 2007

WARNINGS: INCREASED MORTALITY, SERIOUS CARDIOVASCULAR AND THROMBOEMBOLIC EVENTS, and TUMOR PROGRESSION

See full prescribing information for complete boxed warning

Renal failure: **Patients experienced greater risks for death and serious cardiovascular events when administered erythropoiesis-stimulating agents (ESAs) to target higher versus lower hemoglobin levels (13.5 vs. 11.3 g/dL; 14 vs. 10 g/dL) in two clinical studies. Individualize dosing to achieve and maintain hemoglobin levels within the range of 10 to 12 g/dL *[see Warnings and Precautions (5.1)]*.**

Cancer: **Mircera is not indicated for the treatment of anemia due to cancer chemotherapy. A dose-ranging study of Mircera was terminated early because of significantly more deaths among patients receiving Mircera than another ESA. In other studies of ESAs in patients with cancer:**

- **ESAs shortened overall survival and/or time-to-tumor progression in clinical studies in patients with advanced breast, head and neck, lymphoid and non-small cell lung malignancies when dose to a target hemoglobin of ≥ 12 g/dL.**
- **The risks of shortened survival and tumor promotion have not been excluded when ESAs are dosed to target a hemoglobin of < 12 g/dL *[see Warnings and Precautions (5.1)]*.**

----------INDICATIONS AND USAGE----------

Mircera is an erythropoiesis-stimulating agent (ESA) indicated for the treatment of anemia associated with chronic renal failure, including patients on dialysis and patients not on dialysis (1).

Mircera is not indicated for the treatment of anemia due to cancer chemotherapy (1).

----------DOSAGE AND ADMINISTRATION----------

Mircera is administered by subcutaneous (SC) or intravenous (IV) injection (2.1).

- Initial Treatment: 0.6 mcg/kg body weight administered once every two weeks (2.2).
- Conversion from Another ESA: dosed once monthly or once every two weeks based on total weekly Epoetin alfa or Darbepoetin alfa dose at time of conversion (2.2).
- When Mircera is initiated or the dose adjusted: monitor hemoglobin every two weeks until stabilized, and every two to four weeks thereafter (2.1).
- Reduce the dose of Mircera by approximately 25% if: (2.3)
  - rate of rise in hemoglobin is greater than 1 g/dL in 2 weeks
  - hemoglobin is increasing and approaching 12 g/dL

----------DOSAGE FORMS AND STRENGTHS----------

- Single use vials containing 50, 100, 200, 300, 400, 600 or 1000 mcg in 1 mL solution of Mircera (3).
- Single use prefilled syringes containing 50, 75, 100, 150, 200, or 250 mcg in 0.3 mL solution of Mircera and 400, 600 or 800 mcg in 0.6 mL solution of Mircera (3).

----------CONTRAINDICATIONS----------

- Uncontrolled hypertension (4).
- History of hypersensitivity to the drug (4).

----------WARNINGS AND PRECAUTIONS----------

- Hypertension: Do not treat patients with uncontrolled hypertension. Monitor blood pressure throughout course of therapy. Adjust dose or stop Mircera as necessary (2.3, 5.3).
- Seizures: During the first several months of therapy, closely monitor blood pressure and the presence of premonitory neurologic symptoms. (5.4).
- Pure Red Cell Aplasia (PRCA): If anti-erythropoietin antibody associated anemia is suspected, discontinue Mircera. (5.5, 5.6, 6.2)
- Serious allergic reactions: Discontinue Mircera treatment if serious reaction occurs (5.8).
- Predialysis patients may require lower maintenance doses of Mircera than patients receiving dialysis (5.9).
- Marginally dialyzed patients may require adjustments in dialysis prescription (5.10).

----------ADVERSE REACTIONS----------

The most common adverse reactions (≥ 5%) are hypertension, diarrhea, nasopharyngitis, headache, and upper respiratory tract infection (6).

To report SUSPECTED ADVERSE REACTIONS, contact Roche at 1-800-526-6367 or FDA at 1-800-FDA-1088 or www.fda.gov/medwatch.

See 17 PATIENT COUNSELING INFORMATION AND MEDICATION GUIDE

Revised: 11/2007

FULL PRESCRIBING INFORMATION: CONTENTS*

*Sections or subsections omitted from the full prescribing information are not listed.

FULL PRESCRIBING INFORMATION

WARNINGS: INCREASED MORTALITY, SERIOUS CARDIOVASCULAR AND THROMBOEMBOLIC EVENTS, and TUMOR PROGRESSION

Renal failure: **Patients experienced greater risks for death and serious cardiovascular events when administered erythropoiesis-stimulating agents (ESAs) to target higher versus lower hemoglobin levels (13.5 vs. 11.3 g/dL; 14 vs. 10 g/dL) in two clinical studies. Individualize dosing to achieve and maintain hemoglobin levels within the range of 10 to 12 g/dL *[see Warnings and Precautions (5.1)]*.**

Cancer: **Mircera is not indicated for the treatment of anemia due to cancer chemotherapy. A dose-ranging study of Mircera was terminated early because of significantly more deaths among patients receiving Mircera than another ESA. In other studies of ESAs in patients with cancer:**

- **ESAs shortened overall survival and/or time-to-tumor progression in clinical studies in patients with advanced breast, head and neck, lymphoid and non-small cell lung malignancies when dose to a target hemoglobin of ≥ 12 g/dL.**
- **The risks of shortened survival and tumor promotion have not been excluded when ESAs are dosed to target a hemoglobin of < 12 g/dL *[see Warnings and Precautions (5.2)]*.**

1 INDICATIONS AND USAGE

Mircera is indicated for the treatment of anemia associated with chronic renal failure (CRF) in adults, including patients on dialysis and not on dialysis.

Mircera is not indicated for the treatment of anemia due to cancer chemotherapy *[see Warnings and Precautions (5.2)]*.

2 DOSAGE AND ADMINISTRATION

2.1 Important Dosing Information

The dose of Mircera should be reduced as the hemoglobin approaches 12 g/dL or increases by more than 1 g/dL in any 2-week period *[see Warnings and Precautions (5.1)]*. During therapy, hematological parameters should be monitored regularly. Individualize dosing to achieve and maintain hemoglobin levels within the range of 10 to 12 g/dL.

Mircera is administered either intravenously (IV) or subcutaneously (SC). The IV route is recommended for patients receiving hemodialysis because the IV route may be less immunogenic *[see Adverse Reactions (6.2)]*. When administered SC, Mircera should be injected in the abdomen, arm or thigh.

2.2 Starting Dose

Patients Not Currently Treated with an ESA

The recommended starting dose of Mircera for the treatment of anemia in adult CRF patients who are not currently treated with an ESA is 0.6 mcg/kg body weight administered as a single IV or SC injection once every two weeks.

Mircera should be dosed to achieve and maintain hemoglobin between 10 and 12 g/dL. Once the hemoglobin has been maintained within this range, Mircera may be administered once monthly using a dose that is twice that of the every-two-week dose and subsequently titrated as necessary.

Patients Currently Treated with an ESA

Mircera can be administered once every two weeks or once monthly to patients whose hemoglobin has been stabilized by treatment with an ESA (see Table 1). The dose of Mircera, given as a single IV or SC injection, should be based on the total weekly ESA dose at the time of conversion.

Table 1 Mircera Starting Doses for Patients Currently Receiving an ESA

Previous Weekly Epoetin alfa Dose (units/week)	**Previous Weekly Darbepoetin alfa Dose (mcg/week)**	**Mircera Dose**	
		Once Monthly (mcg/month)	Once Every Two Weeks (mcg/every two weeks)
< 8000	< 40	120	60
8000 - 16000	40 - 80	200	100
> 16000	> 80	360	180

2.3 Monitoring and Dose Adjustment

When Mircera therapy is initiated or adjusted, the hemoglobin should be monitored every two weeks until stabilized, and every two to four weeks thereafter. For patients whose hemoglobin does not attain a level within the range of 10 to 12 g/dL despite the use of appropriate Mircera dose titrations over a 12-week period:

- Do not administer higher Mircera doses and use the lowest dose that will maintain a hemoglobin level sufficient to avoid the need for recurrent RBC transfusions
- Evaluate and treat for other causes of anemia
- Thereafter, continue to monitor the hemoglobin level and if responsiveness improves, make Mircera dose adjustments as described above; discontinue Mircera if responsiveness does not improve and the patient needs recurrent RBC transfusions *[see Warnings and Precautions (5.6, 5.7, 5.11)]*.

Dose adjustments should not be made more often than once a month. A significant change in hemoglobin may not be observed for several weeks after the dose is adjusted. If a dose adjustment is necessary to maintain the recommended hemoglobin level, the dose may be increased or decreased by approximately 25%, as needed.

During Mircera therapy, if the increase in hemoglobin is greater than 1 g/dL in 2 weeks or if the hemoglobin is increasing and approaching 12 g/dL, the dose should be reduced by approximately 25%. If the hemoglobin continues to increase, Mircera should be discontinued until the hemoglobin begins to decrease. Mircera may then be restarted at a dose approximately 25% below the previously administered dose.

For patients not converted from another ESA, if the increase in hemoglobin is less than 1 g/dL over the initial 4 weeks of treatment and iron stores are adequate, the dose of Mircera may be increased by approximately 25% *[see Warnings and Precautions (5.11)]*.

If a dose of Mircera is missed, administer the missed dose as soon as possible and restart Mircera at the prescribed dosing frequency.

2.4 Preparation and Administration of Mircera

Mircera is packaged as single use vials and prefilled syringes. Mircera contains no preservatives. Discard any unused portion. Do not pool unused portions from the vials or prefilled syringes. Do not use the vial or prefilled syringe more than one time.

Always store Mircera vials or prefilled syringes in their original cartons. Vigorous shaking or prolonged exposure to light should be avoided.

Do not mix Mircera with any parenteral solution.

Parenteral drug products should be inspected visually for particulate matter and coloration prior to administration. Do not use any vials or prefilled syringes exhibiting particulate matter or a coloration other than colorless to slightly yellowish.

For administration using the prefilled syringe, the plunger must be fully depressed during injection in order for the needle guard to activate. Following administration, remove the needle from the injection site and then release the plunger to allow the needle guard to move up until the entire needle is covered.

See "Patient Instructions for Use" for complete instructions on the preparation and administration of Mircera. Examine each vial or prefilled syringe for the expiration date. Do not use Mircera after the expiration date.

3 DOSAGE FORMS AND STRENGTHS

Single use vials are available containing 50, 100, 200, 300, 400, 600 or 1000 mcg of Mircera in 1 mL solution.

Single use prefilled syringes are available containing 50, 75, 100, 150, 200, or 250 mcg of Mircera in 0.3 mL solution and 400, 600 or 800 mcg of Mircera in 0.6 mL solution.

4 CONTRAINDICATIONS

Mircera is contraindicated in patients with uncontrolled hypertension *[see Warnings and Precautions (5.3)]*.

Mircera is contradicted in patients with a history of hypersensitivity or allergy to the drug *[see Warnings and Precautions (5.8)]*.

5 WARNINGS AND PRECAUTIONS

5.1 Increased Mortality, Serious Cardiovascular And Thromboembolic Events

Anemia associated with chronic renal failure

Patients experienced greater risks for death and serious cardiovascular events when administered ESAs to target higher versus lower hemoglobin levels (13.5 vs. 11.3 g/dL; 14 vs. 10 g/dL) in two clinical studies. Patients with chronic renal failure and an insufficient hemoglobin response to ESA therapy may be at even greater risk for cardiovascular events and mortality than other patients. These events included myocardial infarction, stroke, congestive heart failure, and hemodialysis vascular access thrombosis. A rate of hemoglobin rise of > 1 g/dL over 2 weeks may contribute to these risks.

In a randomized prospective trial, 1432 anemic chronic renal failure patients who were not undergoing dialysis were assigned to Epoetin alfa treatment targeting a maintenance hemoglobin concentration of 13.5 g/dL or 11.3 g/dL. A major cardiovascular event (death, myocardial infarction, stroke, or hospitalization for congestive heart failure) occurred among 125 (18%) of the 715 patients in the higher hemoglobin group compared to 97 (14%) among the 717 patients in the lower hemoglobin group (HR 1.3, 95% CI: 1.0, 1.7 p=0.03).

Increased risk for serious cardiovascular events was also reported from a randomized, prospective trial of 1265 hemodialysis patients with clinically evident cardiac disease (ischemic heart disease or congestive heart failure). In this trial, patients were assigned to Epoetin alfa treatment targeted to a maintenance hemoglobin of either 14 ± 1 g/dL or 10 ± 1 g/dL. Higher mortality (35% vs. 29%) was observed in the 634 patients randomized to a target hemoglobin of 14 g/dL than in the 631 patients randomized to a target hemoglobin of 10 g/dL. The reason for the increased mortality observed in this study is unknown; however, the incidence of nonfatal myocardial infarction, vascular access thrombosis, and other thrombotic events was also higher in the group randomized to a target hemoglobin of 14 g/dL.

Anemia due to other conditions

The safety and efficacy of Mircera have not been established for use among patients with anemia due to cancer chemotherapy or for reduction in the need for allogeneic RBC transfusion in the peri-surgical setting. In these conditions, clinical trials of ESAs have shown risks for thrombotic events and/or mortality.

In a randomized controlled study (referred to as Cancer Study 1 - the "BEST" study) with another ESA in 939 women with metastatic breast cancer receiving chemotherapy, patients received either weekly Epoetin alfa or placebo for up to a year. This study was designed to show that survival was superior when an ESA was administered to prevent anemia (maintain hemoglobin levels between 12 and 14 g/dL or hematocrit between 36% and 42%). The study was terminated prematurely when interim results demonstrated that a higher mortality at 4 months (8.7% vs. 3.4%) and a higher rate of fatal thrombotic events (1.1% vs. 0.2%) in the first 4 months of the study were observed among patients treated with Epoetin alfa. Based on Kaplan-Meier estimates, at the time of study termination, the 12-month survival was lower in the Epoetin alfa group than in the placebo group (70% vs. 76%; HR 1.37, 95% CI: 1.07, 1.75, p=0.012).

A systematic review of 57 randomized controlled trials (including Cancer Studies 1 and 3 - the "BEST" and "ENHANCE" studies) evaluating 9353 patients with cancer compared ESAs plus RBC transfusion with RBC transfusion alone for prophylaxis or treatment of anemia in cancer patients with or without concurrent antineoplastic therapy. An increased relative risk (RR) of thromboembolic events (RR 1.67, 95% CI: 1.35, 2.06; 35 trials and 6769 patients) was observed in ESA-treated patients. An overall survival hazard ratio of 1.08 (95% CI: 0.99, 1.18; 42 trials and 8167 patients) was observed in ESA-treated patients.

An increased incidence of deep vein thrombosis (DVT) in patients receiving Epoetin alfa undergoing surgical orthopedic procedures has been observed. In a randomized controlled study (referred to as the "SPINE" study), 681 adult patients, not receiving prophylactic anticoagulation and undergoing spinal surgery, received Epoetin alfa and standard of care (SOC) treatment, or SOC treatment alone. Preliminary analysis showed a higher incidence of DVT, determined by either Color Flow Duplex Imaging or by clinical symptoms, in the Epoetin alfa group [16 patients (4.7%)] compared to the SOC group [7 patients (2.1%)]. In addition, 12 patients in the Epoetin alfa group and 7 patients in the SOC group had other thrombotic vascular events.

Increased mortality was observed in a randomized placebo-controlled study of Epoetin alfa in adult patients who were undergoing coronary artery bypass surgery (7 deaths in 126 patients randomized to Epoetin alfa versus no deaths among 56 patients receiving placebo). Four of these deaths occurred during the period of study drug administration and all four deaths were associated with thrombotic events.

5.2 Increased Mortality and/or Tumor Progression

A dose-ranging trial of Mircera in 153 patients who were undergoing chemotherapy for non-small cell lung cancer was terminated prematurely because significantly more deaths occurred among patients receiving Mircera than another ESA.

Erythropoiesis-stimulating agents, when administered to target a hemoglobin of > 12 g/dL, shortened the time to tumor progression in patients with advanced head and neck cancer receiving radiation therapy [Cancer Studies 3 and 4 (DAHANCA 10) in Table 2]. ESAs also shortened survival in patients with metastatic breast cancer (Cancer Study 1) and in patients with lymphoid malignancy (Cancer Study 2) receiving chemotherapy when administered to target a hemoglobin of ≥ 12 g/dL. In addition, ESAs shortened survival in patients with non-small cell lung cancer and in a study enrolling patients with various malignancies who were not receiving chemotherapy or radiotherapy; in these two studies, ESAs were administered to target a hemoglobin of ≥ 12 g/dL (Cancer Studies 5 and 6 in Table 2). Although studies evaluated hemoglobin targets of ≥ 12 g/dL in these tumor types, the risks of shortened survival and tumor progression have not been excluded when ESAs are dosed to target a hemoglobin of < 12 g/dL.

Table 2 Randomized, Controlled Trials with Decreased Survival and/or Decreased Locoregional Control

Study/Tumor (n)	Hemoglobin Target	Achieved Hemoglobin (Median Q1,Q3)	Primary Endpoint	Adverse Outcome for ESA-containing Arm
Chemotherapy				
Cancer Study 1 Metastatic breast cancer (n=939)	12-14 g/dL	12.9 g/dL 12.2, 13.3 g/dL	12-month overall survival	Decreased 12-month survival
Cancer Study 2 Lymphoid malignancy (n=344)	13-15 g/dL (M) 13-14 g/dL (F)	11.0 g/dL 9.8, 12.1 g/dL	Proportion of patients achieving a hemoglobin response	Decreased overall survival
Radiotherapy Alone				
Cancer Study 3 Head and neck cancer (n=351)	>15 g/dL (M) >14 g/dL (F)	Not available	Locoregional progression-free survival (LRPFS)	Decreased 5-year locoregional progression-free survival Decreased overall survival
Cancer Study 4 Head and neck cancer (n=522)	14-15.5 g/dL	Not available	Locoregional disease control (LRC)	Decreased locoregional disease control
No Chemotherapy or Radiotherapy				
Cancer Study 5 Non-small cell lung cancer (n=70)	12-14 g/dL	Not available	Quality of life	Decreased overall survival
Cancer Study 6 Non-myeloid malignancy (n=989)	12-13 g/dL	10.6 g/dL 9.4, 11.8 g/dL	RBC transfusions	Decreased overall survival

Decreased overall survival:

Cancer Study 1 (the "BEST" study) was previously described *[see Warnings and Precautions (5.1)]*. Mortality at 4 months (8.7% vs. 3.4%) was significantly higher in the Epoetin alfa arm. The most common investigator-attributed cause of death within the first 4 months was disease progression; 28 of 41 deaths in the Epoetin alfa arm and 13 of 16 deaths in the placebo arm were attributed to disease progression. Investigator assessed time to tumor progression was not different between the two groups. Survival at 12 months was significantly lower in the Epoetin alfa arm (70% vs. 76%, HR 1.37, 95% CI: 1.07, 1.75; p=0.012).

Cancer Study 2 was a Phase 3, double-blind, randomized (Darbepoetin alfa vs. placebo) study conducted in 344 anemic patients with lymphoid malignancy receiving chemotherapy. With a median follow-up of 29 months, overall mortality rates were significantly higher among patients randomized to Darbepoetin alfa as compared to placebo (HR 1.36, 95% CI: 1.02, 1.82).

Cancer Study 5 was a Phase 3, multicenter, randomized (Epoetin alfa vs. placebo), double-blind study, in which patients with advanced non-small cell lung cancer receiving only palliative radiotherapy or no active therapy were treated with Epoetin alfa to achieve and maintain hemoglobin levels between 12 and 14 g/dL. Following an interim analysis of 70 of 300 patients planned, a significant difference in survival in favor of the patients on the placebo arm of the trial was observed (median survival 63 vs. 129 days; HR 1.84; p=0.04).

Cancer Study 6 was a Phase 3, double-blind, randomized (Darbepoetin alfa vs. placebo), 16-week study in 989 anemic patients with active malignant disease, neither receiving nor planning to receive chemotherapy or radiation therapy. There was no evidence of a statistically significant reduction in proportion of patients receiving RBC transfusions. The median survival was shorter in the Darbepoetin alfa treatment group (8 months) compared with the placebo group (10.8 months); HR 1.30, 95% CI: 1.07, 1.57.

Decreased locoregional progression-free survival and overall survival:

Cancer Study 3 (the "ENHANCE" study) was a randomized controlled study in 351 head and neck cancer patients where Epoetin beta or placebo was administered to achieve target hemoglobins of 14 and 15 g/dL for women and men, respectively. Locoregional progression-free survival was significantly shorter in patients receiving Epoetin beta (HR 1.62, 95% CI: 1.22, 2.14, p=0.0008) with a median of 406 days Epoetin beta vs. 745 days placebo. Overall survival was significantly shorter in patients receiving Epoetin beta (HR 1.39, 95% CI: 1.05, 1.84; p=0.02).

Decreased locoregional control:

Cancer Study 4 (DAHANCA 10) was conducted in 522 patients with primary squamous cell carcinoma of the head and neck receiving radiation therapy randomized to Darbepoetin alfa with radiotherapy or radiotherapy alone. An interim analysis on 484 patients demonstrated that locoregional control at 5 years was significantly shorter in patients receiving Darbepoetin alfa (RR 1.44, 95% CI: 1.06, 1.96; p=0.02). Overall survival was shorter in patients receiving Darbepoetin alfa (RR 1.28, 95% CI: 0.98, 1.68; p=0.08).

5.3 Hypertension

Blood pressure should be controlled adequately before initiation of Mircera therapy. Special care should be taken to closely monitor and control blood pressure during Mircera therapy, especially in patients with a history of cardiovascular disease or hypertension. If blood pressure is difficult to control by pharmacologic or dietary measures, the dose of Mircera should be reduced or withheld.

In Mircera clinical studies, approximately 27% of patients with CRF, including patients on dialysis and not on dialysis, required intensification of antihypertensive therapy. Hypertensive encephalopathy and/or seizures have been observed in patients with CRF treated with Mircera *[see Warnings and Precautions (5.4)]*.

5.4 Seizures

Seizures have occurred in patients participating in Mircera clinical studies. During the first several months of therapy, blood pressure and the presence of premonitory neurologic symptoms should be monitored closely. While the relationship between seizures and the rate of rise of hemoglobin is uncertain, the dose of Mircera should be decreased or withheld if the hemoglobin increases more than 1 g/dL in any 2-week period *[see Dosage and Administration (2.3)]*.

5.5 Pure Red Cell Aplasia

Pure red cell aplasia (PRCA) and severe anemia, with or without other cytopenias, have been associated with the development of neutralizing antibodies to erythropoietin in patients treated with ESAs. PRCA occurred predominantly in patients with CRF receiving an ESA by SC administration. PRCA was not observed in clinical studies of Mircera.

Any patient who develops a sudden loss of response to Mircera, accompanied by severe anemia and low reticulocyte count, should be evaluated for the etiology of the altered hemoglobin response, including evaluation for the development of neutralizing antibodies to erythropoietin *[see Warnings and Precautions (5.6)]*. Serum samples should be obtained at least a month after the last Mircera administration to prevent interference of Mircera with the assay. If anti-erythropoietin antibody-associated anemia is suspected, withhold Mircera and other erythropoietic proteins. Contact Roche at 1-800-526-6367 to perform assays for antibodies.

Mircera should be permanently discontinued in patients with antibody-mediated anemia. Patients should not be switched to other erythropoietic proteins as antibodies may cross-react *[see Adverse Reactions (6.2)]*.

5.6 Lack or Loss of Response to Mircera

The lack of a hemoglobin response or failure to maintain a hemoglobin response with Mircera doses within the recommended dosing range should prompt a search for causative factors. Deficiencies of iron, folic acid and vitamin B_{12} should be excluded or corrected.

Intercurrent infections, malignancy, inflammation, occult blood loss, hemolysis, severe aluminum toxicity, osteitis fibrosis cystica, underlying hematological disease (e.g., thalassemia, refractory anemia or myelodysplastic disorders) or bone marrow fibrosis, may also compromise the hemoglobin response. In the absence of another etiology, the patient should be evaluated for evidence of PRCA, including tests for the presence of antibodies to erythropoietin *[see Warnings and Precautions (5.5)]*.

5.7 Hematologic Effects

Sufficient time should be allowed to determine a patient's response to a Mircera dose before adjusting the subsequent doses. Because of the time required for erythropoiesis and the red blood cell (RBC) life span, an interval of 2 to 6 weeks may occur between the time of a dose adjustment (initiation, increase, decrease, or discontinuation) and a significant change in hemoglobin. In order to prevent the hemoglobin from exceeding 12 g/dL or rising too rapidly (greater than 1 g/dL in 2 weeks), the guidelines for dose and frequency of dose adjustments should be followed *[see Dosage and Administration (2.3)]*.

Average platelet counts decreased approximately 7% among patients receiving Mircera in clinical studies with most patients maintaining platelet counts within normal levels. The decrease in platelet counts occurred immediately following Mircera initiation and the levels remained stable thereafter. At least one post-baseline platelet count below 100 x 10^9/L was observed in 7.5% of patients treated with Mircera and 4.4% of patients treated with another ESA.

5.8 Allergic Reactions

Serious allergic reactions, consisting of tachycardia, pruritus and rash, have been reported in patients treated with Mircera. If a serious allergic or anaphylactic reaction occurs due to Mircera, treatment should be immediately and permanently discontinued and appropriate therapy should be administered.

5.9 Patients with CRF Not Requiring Dialysis

Patients with CRF not requiring dialysis may require lower maintenance doses of Mircera than patients receiving dialysis. Patients who are not receiving dialysis may be more responsive to the effects of Mircera and require judicious monitoring of blood pressure and hemoglobin. Renal function and fluid electrolyte balance should also be closely monitored.

5.10 Dialysis Management

Therapy with Mircera results in an increase in red blood cells and a decrease in plasma volume, which could reduce dialysis efficiency; patients who are marginally dialyzed may require adjustments in their dialysis prescription.

5.11 Laboratory Monitoring

In order to ensure effective erythropoiesis, iron status should be evaluated for all patients before and during treatment. Provide supplemental iron therapy for patients whose serum ferritin is below 100 mcg/L or whose serum transferrin saturation is below 20%.

During Mircera therapy, monitor hemoglobin every two weeks until the hemoglobin level has stabilized between 10 and 12 g/dL and the maintenance Mircera dose has been established. The hemoglobin should then

be monitored at least monthly. If a patient requires a dose adjustment or is switched to Mircera from another ESA, monitor hemoglobin every two weeks until the hemoglobin level has stabilized *[see Dosage and Administration (2.1)]*.

6 ADVERSE REACTIONS

The following serious adverse reactions are discussed in greater detail in other sections of the labeling:

- Increased mortality, serious cardiovascular and thromboembolic events *[see Warnings and Precautions (5.1)]*
- Increased mortality and/or tumor progression *[see Warnings and Precautions (5.2)]*
- Hypertension *[see Warnings and Precautions (5.3)]*
- Seizures *[see Warnings and Precautions (5.4)]*
- Pure red cell aplasia *[see Warnings and Precautions (5.5)]*

The most commonly reported adverse reactions were hypertension *[see Warnings and Precautions (5.3)]*, diarrhea, nasopharyngitis, headache, and upper respiratory tract infection. The most common adverse reactions that led to treatment discontinuation in the Mircera clinical studies were: hypertension, coronary artery disease, anemia, concomitant termination of other chronic renal failure therapy and septic shock.

6.1 Clinical Trials Experience

The data described below reflect exposure to Mircera in 2737 patients, including 1451 exposed for 6 months and 1144 exposed for greater than one year. Mircera was studied primarily in active-controlled studies (n=1789 received Mircera, and n=948 received another ESA) and in long-term follow up studies. The population was 18 to 92 years of age, 58% male, and the percentage of Caucasian, Black (including African Americans), Asian and Hispanic patients were 73%, 20%, 5%, and 9%, respectively. Approximately 85% of the patients were receiving dialysis. Most patients received Mircera using dosing regimens of once every two or four weeks, administered SC or IV.

Because clinical trials are conducted under widely varying conditions, adverse reaction rates observed in the clinical studies of Mircera cannot be directly compared to rates in the clinical trials of other drugs and may not reflect the rates observed in practice.

Some of the adverse reactions reported are typically associated with CRF, or recognized complications of dialysis, and may not necessarily be attributable to Mircera therapy. Adverse reaction rates did not importantly differ between patients receiving Mircera or another ESA.

Table 3 summarizes the most frequent adverse reactions (≥ 5%) in patients treated with Mircera.

Table 3 Adverse Reactions Occurring in ≥ 5% of CRF Patients

Adverse Reaction	Patients Treated with Mircera (n=1789)
VASCULAR	
Hypertension	13%
Hypotension	5%
GASTROINTESTINAL	
Diarrhea	11%
Vomiting	6%
Constipation	5%
INFECTIONS AND INFESTATIONS	
Nasopharyngitis	11%
Upper Respiratory Tract Infection	9%
Urinary Tract Infection	5%
NERVOUS SYSTEM	
Headache	9%
MUSCULOSKELETAL AND CONNECTIVE TISSUE	
Muscle Spasms	8%
Back Pain	6%
Pain in Extremity	5%
INJURY, POISONING AND PROCEDURAL COMPLICATIONS	
Procedural Hypotension	8%
Arteriovenous Fistula Thrombosis	5%
Arteriovenous Fistula Site Complication	5%
METABOLISM AND NUTRITION	
Fluid Overload	7%
RESPIRATORY, THORACIC AND MEDIASTINAL	
Cough	6%

In the controlled trials, the rates of serious adverse reactions did not importantly differ between patients receiving Mircera and another ESA (38% vs. 42%) except for the occurrence of serious gastrointestinal hemorrhage (1.2% vs. 0.2%). Serious hemorrhagic adverse reactions of all types occurred among 5% and 4% of patients receiving Mircera or another ESA, respectively.

6.2 Immunogenicity

As with all therapeutic proteins, there is a potential for immunogenicity. Neutralizing antibodies to erythropoietin, in association with PRCA or severe anemia (with or without other cytopenias), have been reported in patients receiving other ESAs during post-marketing experience *[see Warnings and Precautions (5.5)]*. Compared to SC administration, the IV route of administration may lessen the risk for development of antibodies to Mircera.

In 1789 patients treated with Mircera in clinical studies, antibody testing using an enzyme-linked immunosorbent assay (ELISA) was conducted at baseline and during treatment. Antibody development was not detected in any of the patients.

The incidence of antibody formation is highly dependent on the sensitivity and specificity of the assay. Additionally, the observed incidence of antibody (including neutralizing antibody) positivity in an assay may be influenced by several factors including assay methodology, sample handling, timing of sample collection,

concomitant medications, and underlying disease. For these reasons, comparison of the incidence of antibodies to Mircera with the incidence of antibodies to other ESAs may be misleading.

7 DRUG INTERACTIONS

No formal drug/drug interaction studies have been performed.

8 USE IN SPECIFIC POPULATIONS

8.1 Pregnancy: Category C

When Mircera was administered subcutaneously to rats and rabbits during gestation, bone malformation was observed in both species at 50 mcg/kg once every three days. This effect was observed as missing caudal vertebrae resulting in a thread-like tail in one rat fetus, absent first digit metacarpal and phalanx on each forelimb resulting in absent polex in one rabbit fetus, and fused fourth and fifth cervical vertebrae centra in another rabbit fetus. Dose-related reduction in fetal weights was observed in both rats and rabbits. At doses 5 mcg/kg once every three days and higher, Mircera caused exaggerated pharmacodynamic effects in dams. Once-weekly doses of Mircera up to 50 mcg/kg/dose given to pregnant female rats did not adversely affect pregnancy parameters, natural delivery or litter observations. Increased deaths and significant reduction in growth rate of F1 generation were observed during lactation and early post weaning period. However, no remarkable effect on reflex, physical and cognitive development or reproductive performance was observed in F1 generation of any dose groups.

There are no adequate and well-controlled studies in pregnant women. Mircera should be used during pregnancy only if the potential benefit justifies the potential risk to the fetus.

8.3 Nursing Mothers

It is not known whether Mircera is excreted into human breast milk. In one study in rats, Mircera was excreted into maternal milk. Because many drugs are excreted in human milk, caution should be exercised when Mircera is administered to a nursing woman.

8.4 Pediatric Use

The safety and efficacy of Mircera in pediatric patients have not been established.

8.5 Geriatric Use

Clinical studies of Mircera did not include sufficient numbers of subjects aged 65 and over to determine whether they respond differently from younger subjects. Other reported clinical experience has not identified differences in responses between the elderly and younger patients. In general, dose selection for an elderly patient should be cautious, usually starting at the low end of the dosing range, reflecting the greater frequency of decreased hepatic, renal, or cardiac function and of concomitant disease or other drug therapy.

10 OVERDOSAGE

The expected manifestations of Mircera overdosage include signs and symptoms associated with an excessive and/or rapid increase in hemoglobin concentration, including any of the cardiovascular events described above *[see Warnings and Precautions (5.1)]* and *[Adverse Reactions (6.1)]*. Patients receiving an overdosage of Mircera should be monitored closely for cardiovascular events and hematologic abnormalities. Polycythemia should be managed acutely with phlebotomy, as clinically indicated. Following resolution of the effects due to Mircera overdosage, reintroduction of Mircera therapy should be accompanied by close monitoring for evidence of rapid increases in hemoglobin concentration (> 1 g/dL per 14 days). In patients with an excessive hematopoietic response, reduce the Mircera dose in accordance with the recommendations described in *Dosage and Administration (2.3)*.

11 DESCRIPTION

Mircera, methoxy polyethylene glycol-epoetin beta, is an ESA which differs from erythropoietin through formation of a chemical bond between either the N-terminal amino group or the ε-amino group of any lysine present in erythropoietin, predominantly Lys^{52} and Lys^{45} and methoxy polyethylene glycol (PEG) butanoic acid (approximately 30,000 daltons). This results in a total molecular weight of approximately 60,000 daltons. Mircera is formulated as a sterile, preservative-free protein solution for IV or SC administration.

Injectable solutions of Mircera in vials and prefilled syringes are formulated in an aqueous solution containing sodium phosphate, sodium sulphate, mannitol, methionine and poloxamer 188. The solution is clear, colorless to slightly yellowish and the pH is 6.2 ± 0.2.

12 CLINICAL PHARMACOLOGY

12.1 Mechanism of Action

Mircera is an erythropoietin receptor activator with greater activity *in vivo* as well as increased half-life, in contrast to erythropoietin. A primary growth factor for erythroid development, erythropoietin is produced in the kidney and released into the bloodstream in response to hypoxia. In responding to hypoxia, erythropoietin interacts with erythroid progenitor cells to increase red cell production. Production of endogenous erythropoietin is impaired in patients with chronic renal failure (CRF) and erythropoietin deficiency is the primary cause of their anemia.

12.2 Pharmacodynamics

Following a single dose of Mircera in CRF patients, the onset of hemoglobin increase (defined as an increase > 0.4 g/dL from baseline) was observed 7 to 15 days following initial dose administration *[see Dosage and Administration (2.3)]*.

12.3 Pharmacokinetics

The pharmacokinetics of Mircera were studied in anemic patients with CRF including patients on dialysis and not on dialysis. Mircera pharmacokinetics, based on population analyses, were not altered by age, gender, race, or the use of dialysis.

Following an IV administration of Mircera 0.4 mcg/kg body weight to CRF patients receiving peritoneal dialysis, the observed terminal half-life was 134 ± 65 hours (mean ± SD), and the total systemic clearance was 0.49 ± 0.18 mL/hr/kg. Following a SC administration of Mircera 0.8 mcg/kg to CRF patients receiving peritoneal dialysis, the terminal half-life was 139 ± 67 hours. The maximum serum concentrations of Mircera were observed 72 hours (median value) following the SC administration. The absolute bioavailability of Mircera after the SC administration was 62%.

In CRF patients receiving multiple Mircera doses, pharmacokinetics were studied after the first dose and on week 9 and week 19 or 21. Multiple dosing was found to have no effect on clearance, volume of distribution or bioavailability of Mircera. Based on population analyses of the clinical studies, Mircera did not accumulate following administration every four weeks. However, when Mircera was administered every 2 weeks, blood concentrations at steady state increased by 12%.

A comparison of serum concentrations of Mircera measured before and after hemodialysis in 41 patients showed that hemodialysis did not alter serum concentrations.

The site of SC injection (abdomen, arm or thigh) had no clinically important effects on the pharmacokinetics or pharmacodynamics of Mircera in healthy volunteers.

13 NONCLINICAL TOXICOLOGY

13.1 Carcinogenesis, Mutagenesis, Impairment of Fertility

Carcinogenicity

The carcinogenic potential of Mircera has not been evaluated in long-term animal studies. Mircera did not induce a proliferative response in either the erythropoietin receptor positive cell lines HepG2 and K562 or the erythropoietin receptor negative cell line RT112 *in vitro*. In addition, using a panel of human tissues, the *in vitro* binding of Mircera was observed only in bone marrow progenitor cells.

Mutagenicity

The mutagenic potential of Mircera has not been evaluated.

Impairment of Fertility

When Mircera was administered subcutaneously to male and female rats prior to and during mating, reproductive performance, fertility, and sperm assessment parameters were not affected.

14 CLINICAL STUDIES

The efficacy and safety of Mircera were assessed in six open-label, multi-center clinical studies that randomized patients to either Mircera or a comparator ESA. Two studies evaluated anemic patients with CRF who were not treated with an ESA at baseline and four studies evaluated patients who were receiving an ESA for treatment of the anemia of CRF. In all studies, patients were assessed as clinically stable at baseline and without evidence of infection or inflammation as determined by history and laboratory data, including C-reactive protein (CRP $\leq$ 15 mg/L for study 1 and CRP $\leq$ 30 mg/L for studies 2 to 6). A CRP value above the threshold led to the exclusion of no more than 3% of the screened patients.

In the clinical studies, ESAs were administered to achieve specific hemoglobin levels (see Table 4 and Table 5). Following stabilization of hemoglobin levels (12 g/dL), the median monthly Mircera dose was 150 mcg (range of 97 mcg to 270 mcg).

Patients Not Currently Treated with an ESA

In Study 1 patients who were not receiving dialysis were randomized to Mircera or darbepoetin alfa, administered for 28 weeks. The starting dose of Mircera was 0.6 mcg/kg administered SC once every two weeks and the starting dose of darbepoetin alfa was 0.45 mcg/kg administered SC once a week. In Study 2, patients who were receiving dialysis were randomized to Mircera or another ESA (Epoetin alfa or Epoetin beta), administered for 24 weeks. The starting dose of Mircera was 0.4 mcg/kg administered IV once every two weeks and the starting dose of the comparator was administered IV three times a week, consistent with the product's recommended dose. In these studies, the observed median dose of Mircera once every two weeks over the course of the correction/evaluation period was 0.6 mcg/kg. Table 4 provides the results of the two studies.

Table 4 Clinical Studies in Paticnts Not Currently Treated with an ESA

Group (n)	Percent Achieving Goal* (95% CI)	Mean Hemoglobin Change from Baseline (g/dL)	RBC Transfusion, %
Study 1			
Mircera (n=162)	98 (94, 99)	2.1	2.5
Darbepoetin alfa (n=162)	96 (92, 99)	2.0	6.8
Study 2			
Mircera (n = 135)	93 (88, 97)	2.7	5.2
Epoetin alfa/beta (n=46)	91 (79, 98)	2.6	4.3

*Goal: hemoglobin increase of at least 1 g/dL and to a level of at least 11 g/dL without RBC transfusion; hemoglobin levels were to be maintained within the range of 11 to 13 g/dL.

Patients Currently Treated with an ESA

Four studies assessed the ability of Mircera to maintain hemoglobin concentrations among patients currently treated with other ESAs. Patients were randomized to receive Mircera administrations either once every two weeks or once every four weeks, or to continue their current ESA dose and schedule. The initial Mircera dose was determined based on the patient's previous weekly ESA dose. As shown in Table 5, treatment with Mircera once every two weeks and once every four weeks maintained hemoglobin concentrations within the targeted hemoglobin range (10 to 13.5 g/dL).

Table 5 Clinical Studies in Patients Currently Treated with an ESA

Group (n)	Mean Baseline Hemoglobin	Evaluation Period Hemoglobin (Mean)	Between-group Difference *, g/dL (95% or 97.5% CI)
Study 3			
Mircera IV every 2 weeks (n=223)	12.0	11.9	0.0 (−0.2, 0.2)
Mircera IV every 4 weeks (n=224)	11.9	11.9	0.1 (−0.2, 0.3)
Epoetin alfa/beta IV (n=226)	12.0	11.9	n/a
Study 4			
Mircera SC every 2 weeks (n=190)	11.7	11.7	0.1 (−0.1, 0.4)
Mircera SC every 4 weeks (n=191)	11.6	11.5	−0.0 (−0.3, 0.2)
Epoetin beta SC (n=191)	11.6	11.5	n/a
Study 5			
Mircera, IV every 2 weeks (n=157)	12.0	12.1	0.2 (−0.0, 0.4)
Darbepoetin alfa IV (n=156)	11.9	11.8	n/a

Study 6			
Mircera IV/SC every 2 weeks (n= 68)	11.8	11.9	0.1 (−0.1, 0.4)
Epoetin alfa IV/SC (n=168)	11.9	11.8	0.1 (−0.1, 0.4)

*Mircera versus comparator mean hemoglobin difference in the evaluation period; 97.5% CI are shown for studies that compared two Mircera groups to another ESA (Studies 3 and 4) and 95% CI are shown for the other studies.
n/a = not applicable

16 HOW SUPPLIED/STORAGE AND HANDLING

16.1 How Supplied

Mircera is available in single use vials and single use prefilled syringes. The vial caps and plungers of prefilled syringes are designated with unique colors for each dosage strength. The prefilled syringes are supplied with a 27 gauge, ½ inch needle. To reduce the risk of accidental needlesticks after application, each prefilled syringe is equipped with a needle guard that covers the needle during disposal.

Mircera is available in the following pack sizes:

Single Use Vial:		**Single Use Prefilled Syringe (PFS) with a Needle Guard. A 27 Gauge, ½ Inch Needle is also provided:**
1 Vial/Pack	**12 Vials/Pack**	**1 PFS/Pack**
50 mcg/1 mL (NDC 0004-0411-09)	50 mcg/1 mL (NDC 0004-0411-06)	50 mcg/0.3 mL (NDC 0004-0401-09)
100 mcg/1 mL (NDC 0004-0413-09)	100 mcg/1 mL (NDC 0004-0413-06)	75 mcg/0.3 mL (NDC 0004-0402-09)
200 mcg/1 mL (NDC 0004-0415-09)	200 mcg/1 mL (NDC 0004-0415-06)	100 mcg/0.3 mL (NDC 0004-0403-09)
300 mcg/1 mL (NDC 0004-0417-09)	300 mcg/1 mL (NDC 0004-0417-06)	150 mcg/0.3 mL (NDC 0004-0404-09)
400 mcg/1 mL (NDC 0004-0418-09)	400 mcg/1 mL (NDC 0004-0418-06)	200 mcg/0.3 mL (NDC 0004-0405-09)
600 mcg/1 mL (NDC 0004-0419-09)	600 mcg/1 mL (NDC 0004-0419-06)	250 mcg/0.3 mL (NDC 0004-0406-09)
1000 mcg/1 mL (NDC 0004-0420-09)	1000 mcg/1 mL (NDC 0004-0420-06)	400 mcg/0.6 mL (NDC 0004-0408-09)
		600 mcg/0.6 mL (NDC 0004-0409-09)
		800 mcg/0.6 mL (NDC 0004-0410-09)

16.2 Stability and Storage

The recommended storage temperature is at 2° to 8°C (36°F to 46°F). Do not freeze or shake. Protect from light.

Storage of vials over the recommended temperature (2°C to 8°C), when necessary, is permissible only for temperatures up to 25°C (77°F) and for no more than 7 days.

Storage of prefilled syringes over the recommended temperature (2°C to 8°C), when necessary, is permissible only for temperatures up to 25°C (77°F) and for no more than 30 days.

17 PATIENT COUNSELING INFORMATION

See Medication Guide (17.2) and Patient Instructions for Use

17.1 Information for Patients

Inform patients of the:

- Need for regular blood pressure monitoring and laboratory tests for hemoglobin in order to lessen the risks for mortality and serious cardiovascular events
- Possible side effects of Mircera, including injection site reactions, allergic reactions and the potential problems due to excessive increases in blood hemoglobin levels *[see Warnings and Precautions (5.1]*
- Signs and symptoms of injection site and allergic reactions
- Importance of compliance with any prescribed dietary restrictions, dialysis regimens or medications, including antihypertensive medications

Administer Mircera under the direct supervision of a healthcare provider or, in situations where a patient has been trained to administer Mircera at home, provide instruction on the proper use of Mircera, including instructions to:

- Carefully review the Medication Guide and the Patient Instructions for Use
- Avoid the reuse of needles, syringes, or unused portions of the Mircera vials or prefilled syringes and to properly dispose of these items
- Always keep a puncture-proof disposal container available for the disposal of used syringes and needles

17.2 Medication Guide

MEDICATION GUIDE

MIRCERA® (mir-SER-ah)

(methoxy polyethylene glycol-epoetin beta)

Read this Medication Guide carefully before you start taking Mircera and each time you refill your Mircera prescription. This Medication Guide does not take the place of talking to your healthcare provider about your medical condition or your treatment.

What is the most important information I should know about Mircera?

Mircera stimulates your bone marrow to make more red blood cells. The increase in red blood cells also increases your hemoglobin level. If your hemoglobin level stays too high or if your hemoglobin goes up too quickly, this may lead to serious health problems which may result in death. These problems include:

Serious heart problems. These problems include heart attack, stroke, and congestive heart failure.

Blood clots. Mircera treatment increases your chance of a blood clot. If you are scheduled for surgery,

your healthcare provider may prescribe a blood thinner to prevent blood clots. Blood clots can form in your hemodialysis vascular access (such as arteriovenous fistulas) or in blood vessels, especially in the leg (deep venous thrombosis or DVT). Pieces of a blood clot may travel to the lungs. If this happens, blood circulation in the lungs may be blocked (pulmonary embolus).

Tell your healthcare provider or get medical attention right away if you have any of these symptoms while taking Mircera:

- Chest pain
- Trouble breathing or shortness of breath
- Pain in the legs, with or without swelling
- A cool or pale arm or leg
- Sudden confusion or trouble speaking or understanding speech
- Sudden numbness or weakness of the face, an arm or leg, especially on one side of the body
- Sudden trouble seeing in one or both eyes
- Sudden trouble walking, dizziness, loss of balance or coordination, loss of consciousness
- Sudden severe headache with no known cause
- Seizures (convulsions)
- Blood clots in your hemodialysis vascular access (such as arteriovenous fistulas).

It is important for you to have the blood tests ordered by your healthcare provider. Your healthcare provider will try to keep your hemoglobin level between 10 and 12 g/dL.

Mircera is not used to treat anemia caused by cancer chemotherapy. In patients with cancer, drugs that act like Mircera increase the chance of dying sooner or making the cancer grow faster. In a clinical study of cancer patients, more deaths occurred among patients receiving Mircera compared to another drug that also increases blood hemoglobin.

What is Mircera?

Mircera is a man-made form of the human protein erythropoietin. Erythopoietin is normally produced by the kidneys. Mircera and other man-made erythropoietins are ESAs (Erythropiesis-Stimulating Agents). ESAs stimulate bone marrow to make red blood cells. The increase in red blood cells also increases the blood hemoglobin level. Your healthcare provider will prescribe the lowest dose of Mircera needed to help increase your hemoglobin level to between 10 to 12 g/dL and to help avoid the need for red blood cell transfusions.

You may be asked to have certain blood tests, such as hemoglobin, hematocrit, or iron level measurements. Based on your test results, your healthcare provider will adjust the dose of Mircera as needed to reach the right dose for you and to help prevent serious side effects. The right dose for you may change over time.

Mircera is not used to treat anemia that is caused by other health problems, such as cancer.

Mircera has not been studied in children.

Who should not take Mircera?

Do not take Mircera if:

- You have high blood pressure that is not controlled (uncontrolled hypertension)
- You have allergies to Mircera or other ESAs
- You have anemia caused by cancer chemotherapy

What should I tell my healthcare provider before taking Mircera?

Mircera may not be right for you. Tell your healthcare provider about all of your medical conditions, including if you:

- Have heart disease
- Have or develop cancer
- Have high blood pressure
- Have any history of stroke, blood clots or seizures
- Have blood disorders (such as sickle cell anemia or clotting disorders)
- Are pregnant, think you may be pregnant or plan to become pregnant. The effect of Mircera on pregnant women is unknown. It is also not known if Mircera could harm an unborn baby.
- Are breast-feeding or plan to breast-feed. It is not known if Mircera passes into human breast milk.

Tell your healthcare provider about all of the medicines you take, including prescription and non-prescription medicines, vitamins, and herbal supplements.

How should I take Mircera?

Mircera is taken as either an intravenous (IV) or subcutaneous (SC) injection. It can take two to six weeks of treatment to see an increase in your hemoglobin level. If the desired increase is not seen, your healthcare provider may change your treatment dose.

- Mircera should be administered by your healthcare provider. In some cases, your healthcare provider may allow you or your caregiver to give the injections at home.
- If you or your caregiver are allowed to give the injections at home, it is important that you carefully follow the instructions that your healthcare provider gives you. Be sure that you read, understand, and follow the "Patient Instructions for Use."
- Take Mircera exactly as your healthcare provider tells you to. Do not change the dose of Mircera unless told to by your healthcare provider. Your healthcare provider will show you or your caregiver how much Mircera to use, how to inject it, how often it should be injected and how to safely throw away used needles and syringes.
- Change the skin site for each injection to avoid soreness at any one site. Sometimes a problem may develop at the injection site. If there is a lump, swelling, or bruising at the injection site that does not go away, talk to your healthcare provider.

- **To use Mircera safely at home, it is important that you:**
  - Use the contents of a vial or prefilled syringe one time only
  - Throw away any solution remaining in the vial after use
  - Use a needle and syringe only one time for injection

- If you have a hemodialysis vascular access, regularly check it to make sure it is working. Call your healthcare provider or dialysis center right away if you have any problems or questions.
- If you miss one dose of Mircera, take your dose right away and then continue as you have been told by your healthcare provider. If you miss more than one dose, call your healthcare provider right away for instructions on what to do.

- If you take more than the prescribed amount of Mircera, call your healthcare provider right away for instructions on what to do.
- Continue to follow your healthcare provider's instructions for diet, dialysis, and medicines including medicines for high blood pressure, while taking Mircera.

What are possible side effects of Mircera?

Mircera can cause serious side effects. See **"What is the most important information I should know about Mircera?"**

Other side effects, which may be serious include:

- **High blood pressure.** Your blood pressure may go up when the numbers of red blood cells increase while taking Mircera. This can happen even if you have never had high blood pressure before. Your healthcare provider or caregiver should check your blood pressure often. If you have a history of heart problems or high blood pressure, talk with your healthcare provider about how often to check your blood pressure. Call your healthcare provider if your blood pressure changes from what is normal for you. If your blood pressure does increase, your healthcare provider may prescribe new or more blood pressure medicine.
- **Seizures.** Seizures can occur in people receiving Mircera. If you have any seizures while taking Mircera, get medical help right away and tell your healthcare provider.
- **Serious allergic reaction.** Mircera may cause a serious allergic reaction. Symptoms of a serious allergic reaction may include: a rash all over the body, shortness of breath, wheezing, dizziness, fainting, swelling around the mouth or eyes, fast pulse, or sweating. If a serious allergic reaction occurs, stop using Mircera and call your healthcare provider or get emergency medical help right away.
- **No response or loss of your hemoglobin response to Mircera.** If your hemoglobin does not reach the desired level of 10 to 12 g/dL or your hemoglobin does not stay within this level, your healthcare provider will look for the cause of the problem. Your dose of Mircera or other medicines may need to be changed.
- **Antibodies to Mircera.** Your body may make antibodies to Mircera. These antibodies can block or reduce your body's ability to make red blood cells, and cause you to have severe anemia. Call your healthcare provider if you have unusual tiredness, lack of energy, dizziness or fainting.

The most common side effects you may have when taking Mircera are:

- Increased blood pressure (hypertension)
- Diarrhea
- Upper respiratory tract infections (cold, cough and sinus infections)
- Headache

Other side effects when taking Mircera may include:

- Decreased blood pressure (hypotension)
- Vomiting
- Constipation
- Urinary tract infections
- Body or muscle aches, including back pain
- Swelling in your arms or legs with or without shortness of breath
- Problems with your hemodialysis vascular access (such as arteriovenous fistulas), including clotting and fistula site problems

- Injection site reactions such as redness, swelling, or itching. Tell your healthcare provider if you have any side effect that bothers you or that does not go away.

These are not all the possible side effects of Mircera. Your healthcare provider or pharmacist can give you a more complete list.

How should I store Mircera?

- Keep Mircera in the original package. Protect Mircera from light. Do not use Mircera that has been left in bright light.
- Do not shake Mircera
- Store Mircera in the refrigerator at 36°F to 46°F (2°C to 8°C)
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- Do not freeze Mircera. Do not use Mircera that has been frozen or improperly refrigerated. Talk to your healthcare provider or pharmacist with any questions about storing Mircera.

Keep Mircera and all medicines out of the reach of children.

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What are the ingredients in Mircera?

Active ingredient: methoxy polyethylene glycol-epoetin beta

Inactive ingredients: sodium phosphate, sodium sulphate, mannitol, methionine and poloxamer 188

This Medication Guide has been approved by the U.S. Food and Drug Administration

Hoffmann-La Roche Inc.
340 Kingsland Street
Nutley, New Jersey 07110-1199

U.S. Govt. Lic. No. 0136

Issued: November 2007

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MEDICATION GUIDE

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General Information about Mircera

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Patient Instructions for Use

MIRCERA® (mir-SER-ah)
(methoxy polyethylene glycol-epoetin beta)

NOV 14 2007

Single Use Prefilled Syringe (for Injection)

Read the Medication Guide that comes with Mircera for the most important information you need to know. It is also very important that you carefully read and understand these instructions for how to give yourself the correct dose of Mircera.

When you receive your supply of Mircera, make sure that:

1. The name Mircera appears on the pack.
2. Mircera is used before the expiration date on the pack. Do not use Mircera after the expiration date.
3. The dosage strength on the Mircera pack matches the strength prescribed [number of micrograms (mcg)].
4. The liquid in Mircera is clear and colorless to slightly yellow. Do not use Mircera if the liquid appears discolored or cloudy, or if it appears to have lumps, flakes or particles in it.
5. The needle cover is on the prefilled syringe of Mircera. Do not use the prefilled syringe of Mircera if the cover on the needle is off.
6. The prefilled syringe and needle used for administration of Mircera is the one prescribed by your healthcare provider. The dose of Mircera will be measured in micrograms (mcg). Mircera can be given either by a prefilled syringe or a vial using a syringe and needle. Mircera prefilled syringes and vials come in several different strengths. If you change from using the vials (syringes and needles) of Mircera to the prefilled syringes, the strength of medicine will be different. Talk with your healthcare provider or pharmacist to be sure you understand the difference. **Use the prefilled syringe only once. Throw away the prefilled syringe in a puncture-proof disposable container after use as instructed by your healthcare provider.**

Your healthcare provider should tell you how to give the correct dose of Mircera:

- How much Mircera to use
- How to inject
- How often it should be injected
- How to throw away used syringes

IMPORTANT: FOLLOW THESE INSTRUCTIONS TO GIVE MIRCERA INJECTIONS AND AVOID POSSIBLE INFECTION.

SETTING UP FOR AN INJECTION

1. Find a clean, flat work surface such as a table.
2. Take a pack of Mircera from the refrigerator. Do not freeze Mircera or use a prefilled syringe that has been frozen. Do not shake Mircera or leave it in bright light. Shaking the prefilled syringe or exposing it to light may damage Mircera and it may not work as well. If the Mircera prefilled syringe has been shaken, the solution may look foamy and should not be used.
3. Take the prefilled syringe of Mircera out of its pack and place it on your flat work surface.
4. Use a prefilled syringe only once.
5. Gather the supplies you will need for an injection (see Figure 1). You will need:

- Mircera prefilled syringe with a clear plastic needle guard attached
- One alcohol swab and one cotton ball or gauze
- Puncture-proof disposable container, which will be given to you by your healthcare provider, for safely throwing away the prefilled syringe after injection

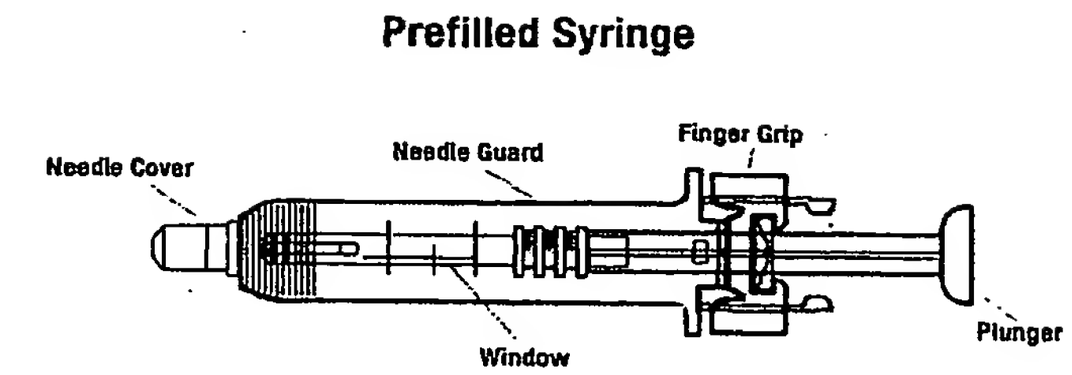

Figure 1.

6. Wash your hands well with soap and warm water before preparing the dose.

PREPARING THE DOSE OF Mircera

1. Open the wrapper and remove the prefilled syringe and needle.

2. Break the seal and remove the plastic cap from the back of the needle (see Figure 2).

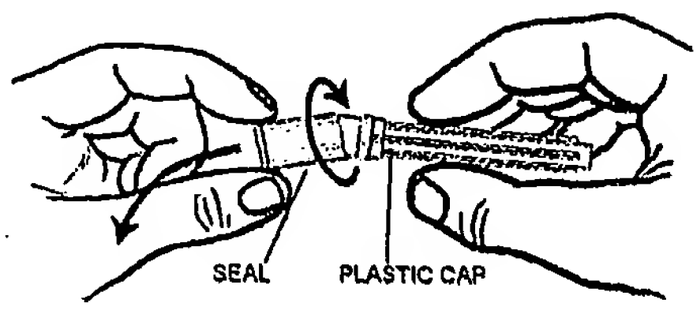

Figure 2.

3. Remove the rubber tip cap from the prefilled syringe. It may require a strong pull (see Figure 3).

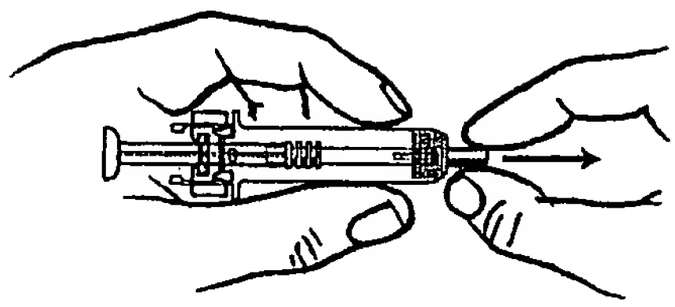

Figure 3.

4. Attach the needle to the prefilled syringe (see Figure 4).

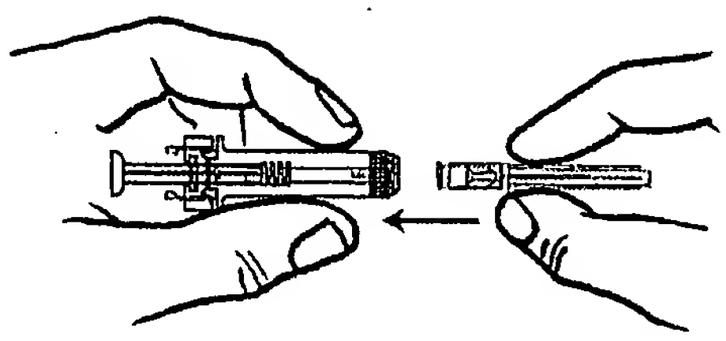

Figure 4.

5. Put the prefilled syringe on its side with the needle cover on. This will keep the needle from touching anything before you use it (see Figure 5).

Figure 5.

5 SELECTING AND PREPARING THE INJECTION SITE

1. Choose an injection site (see Figure 6). The three sites where you can inject Mircera include:

- the outer area of the upper arms
- the front of the middle thighs
- the abdomen (except for the two-inch area around the navel)

Choose a new injection site each time you inject Mircera. This helps to avoid soreness at any one site.

Do not inject Mircera into an area on your body that is tender, red, bruised, hard, or that has scars or stretch marks.

Figure 6.

2. Clean the injection site with a new alcohol swab. Do not touch this area again before giving the injection (see Figure 7).

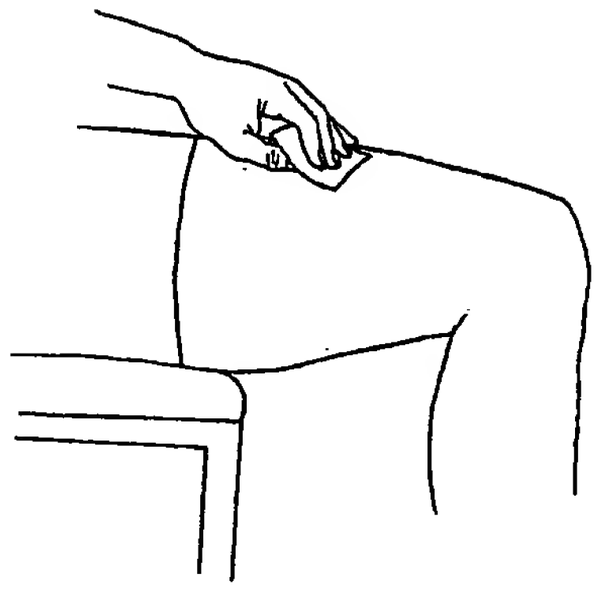

Figure 7.

INJECTING THE DOSE OF Mircera FOR PATIENTS NOT ON HEMODIALYSIS

1. Remove the plastic cover from the needle (see Figure 8).

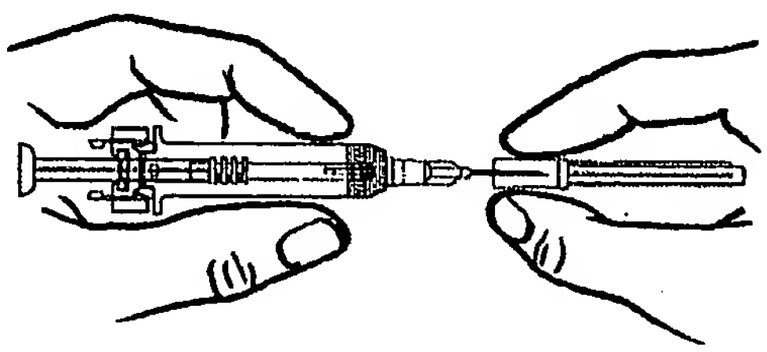

Figure 8.

2. Remove air bubbles from the syringe. Hold the prefilled syringe with the needle pointing up. Tap the prefilled syringe gently to bring the bubbles to the top. Push the plunger up slowly to the correct dose.

3. Hold the prefilled syringe in the hand that you will use to inject Mircera. Use the other hand to pinch a fold of skin at the cleaned injection site (see Figure 9).

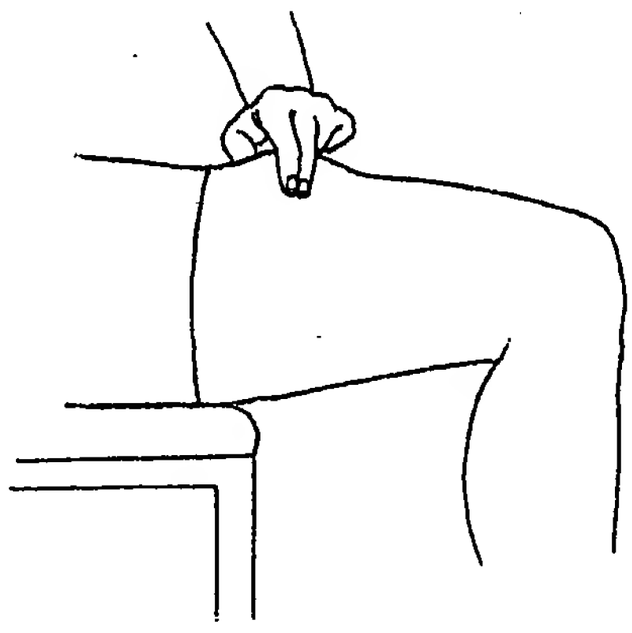

Figure 9.

4. Hold the prefilled syringe like a pencil. Insert the needle in a quick "dart like" motion. Inject either at a slight angle (45 degree angle) or straight up and down (90 degree angle) into the skin (see Figure 10).

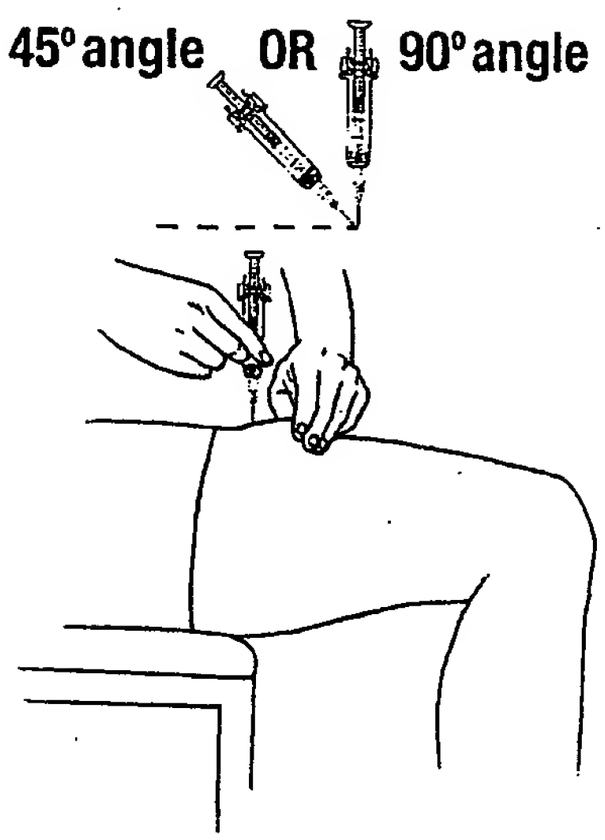

Figure 10.

5. Pull the plunger back slightly after inserting the needle into the skin. If blood comes into the prefilled syringe, do not inject Mircera because the needle has entered a blood vessel. Remove the needle from the skin. Slightly reposition the needle within the cleaned area and repeat. If blood does not come, slowly push the plunger all the way down until all the medicine is injected. The plastic needle guard (a safety mechanism to prevent accidental needle sticks) will not move forward to cover the needle unless the full dose is given. (see Figure 11).

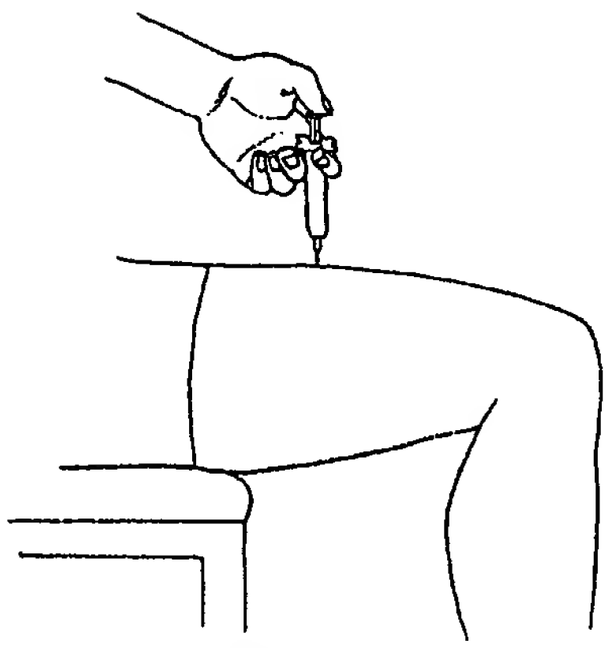

Figure 11.

6. Take the needle out of the skin without releasing the plunger (see Figure 12).

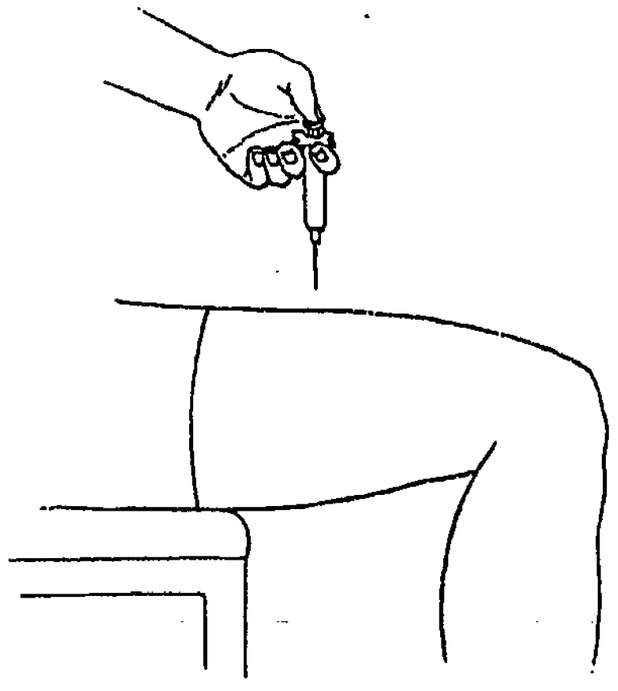

Figure 12.

7. Release the plunger after removing the needle from the skin. This allows the syringe to move back until the entire needle is guarded (see Figure 13).

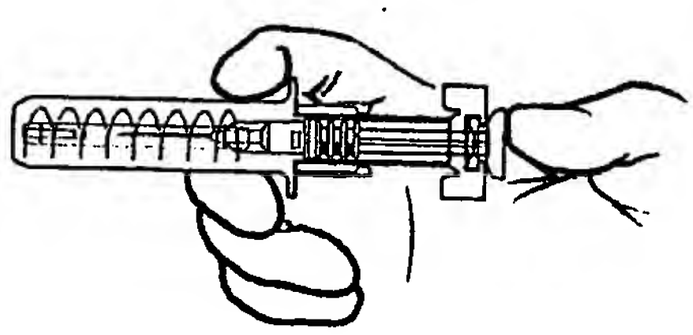

Figure 13.

8. Place a cotton ball or gauze over the injection site and press for several seconds (see Figure 14).

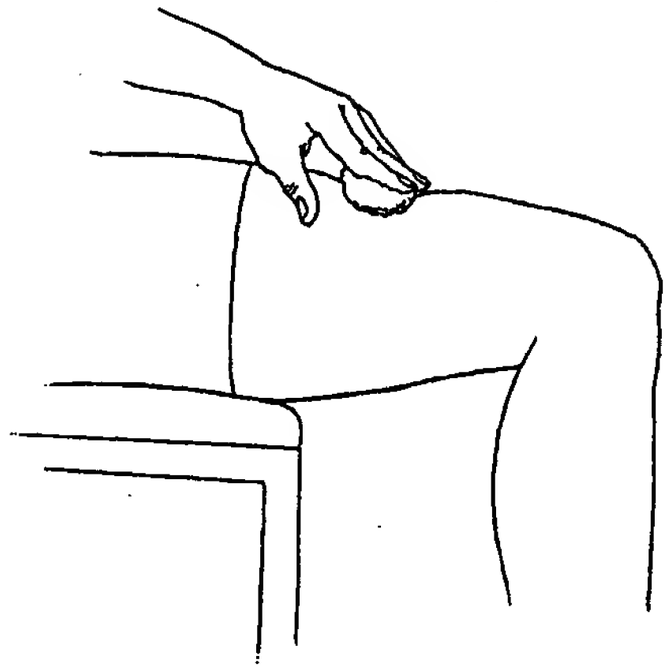

Figure 14.

9. Dispose of the syringe with any remaining liquid in the puncture-proof disposable container. Use the prefilled syringe one time only.

9 **FOR PATIENTS ON HEMODIALYSIS USING VENOUS INJECTION**

1. Clean the venous port of the hemodialysis tubing with a new alcohol swab (see Figure 15).

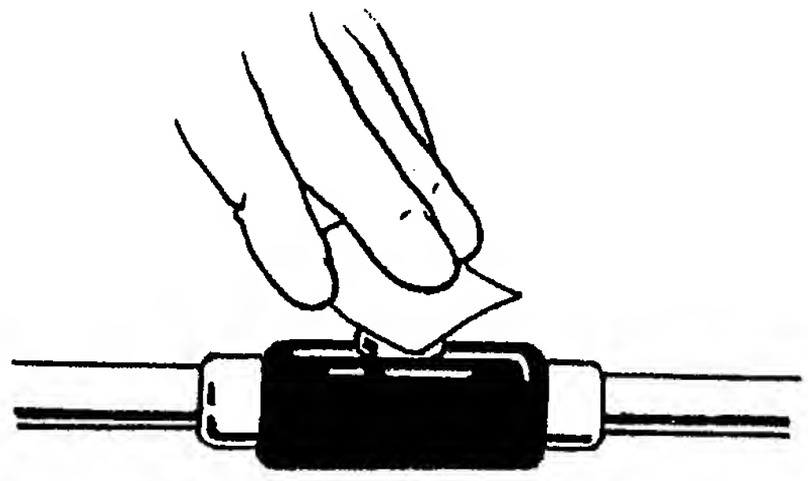

Figure 15.

2. Insert the needle of the syringe into the cleaned venous port and push the plunger all the way down to inject all the medicine (see Figure 16).

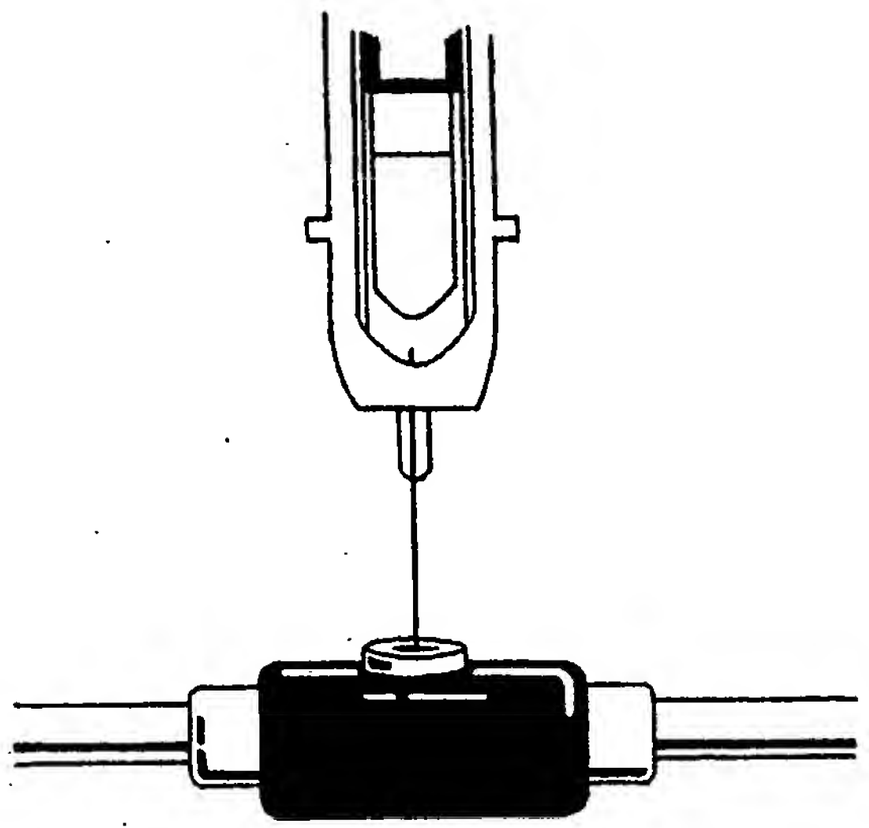

Figure 16.

3. Remove the syringe from the venous port. Dispose of the syringe with any remaining liquid in the puncture-proof disposable container. Use the prefilled syringe one time only.

Disposing of Syringes and Needles

Follow the required state and local laws for disposal of needles and syringes. Ask your healthcare provider or pharmacist about correct disposal of used syringes and needles.

Use the information below as a general guide:

- never re-use a syringe or needle
- place used syringes and needles in the puncture-proof disposable container
- DO NOT use glass or clear plastic containers to throw away syringes and needles
- Throw away the full puncture-proof disposable container as instructed by your healthcare provider or pharmacist

DO NOT throw away the puncture-proof disposable container in your household trash. DO NOT recycle.

Keep the container out of the reach of children.

Hoffmann-La Roche Inc.
340 Kingsland Street
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Issued: November 2007

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Patient Instructions for Use

MIRCERA® (mir-SER-ah)
(methoxy polyethylene glycol-epoetin beta)

Single Use Vial (for Injection)

Read the Medication Guide that comes with Mircera for the most important information you need to know. It is very important that you carefully read and understand these instructions for how to give yourself the correct dose of Mircera.

When you receive your supply of Mircera, make sure that:

1. The name Mircera appears on the pack.
2. Mircera is used before the expiration date on the pack. Do not use Mircera after the expiration date.
3. The dosage strength on the Mircera pack matches the strength prescribed [number of micrograms (mcg)].
4. The liquid in Mircera is clear and colorless to slightly yellow. Do not use Mircera if the liquid appears discolored or cloudy, or if it appears to have lumps, flakes or particles in it.
5. The colored cap is on the vial of Mircera. Do not use the vial of Mircera if the colored cap has been removed.
6. The disposable syringe and needle used for administration of Mircera are the ones prescribed by your healthcare provider. Mircera can be given either by a prefilled syringe or a vial using a syringe and needle. Mircera vials and prefilled syringes come in several different strengths. If you change from using the vials (syringes and needles) of Mircera to the prefilled syringes, the strength of medicine will be different. Talk with your healthcare provider or pharmacist to be sure you understand the difference. **Use the disposable syringe and needle only once. Throw away the syringe and needle in a puncture-proof disposable container after use as instructed by your healthcare provider.**

Your healthcare provider should tell you how to give the correct dose of Mircera:

- How much Mircera to use
- How to inject
- How often it should be injected
- How to throw away used needles and syringes

The dose will be measured in mcg per milliliters (mL). Use only a disposable syringe that is marked in tenths of mL (for example, 0.2 mL). Your healthcare provider may refer to an "mL" as a "cc" (1 mL = 1 cc). Do not use an unmarked syringe. Using an unmarked syringe can lead to a mistake in the dose. If you do not use the correct syringe, you could inject too much or too little Mircera.

IMPORTANT: FOLLOW THESE INSTRUCTIONS TO GIVE MIRCERA INJECTIONS AND AVOID POSSIBLE INFECTION.

SETTING UP FOR AN INJECTION

1. Find a clean, flat work surface such as a table.
2. Take a pack of Mircera from the refrigerator. Do not freeze Mircera or use a vial that has been frozen. Do not shake Mircera or leave it in bright light. Shaking the vial or exposing it to light may damage Mircera and it may not work as well. If the Mircera vial has been shaken, the solution may look foamy and should not be used.
3. Take the vial of Mircera out of its pack and place it on your flat work surface.
4. Use a vial only once. Do not put the needle through the rubber stopper more than once.

5. Gather the supplies you will need for an injection (see Figure 1). You will need:

- Mircera vial and the correct disposable syringe and needle
- Two alcohol swabs and one cotton ball or gauze
- Puncture-proof disposable container, which will be given to you by your healthcare provider, for safely throwing away the needle and syringe after injection

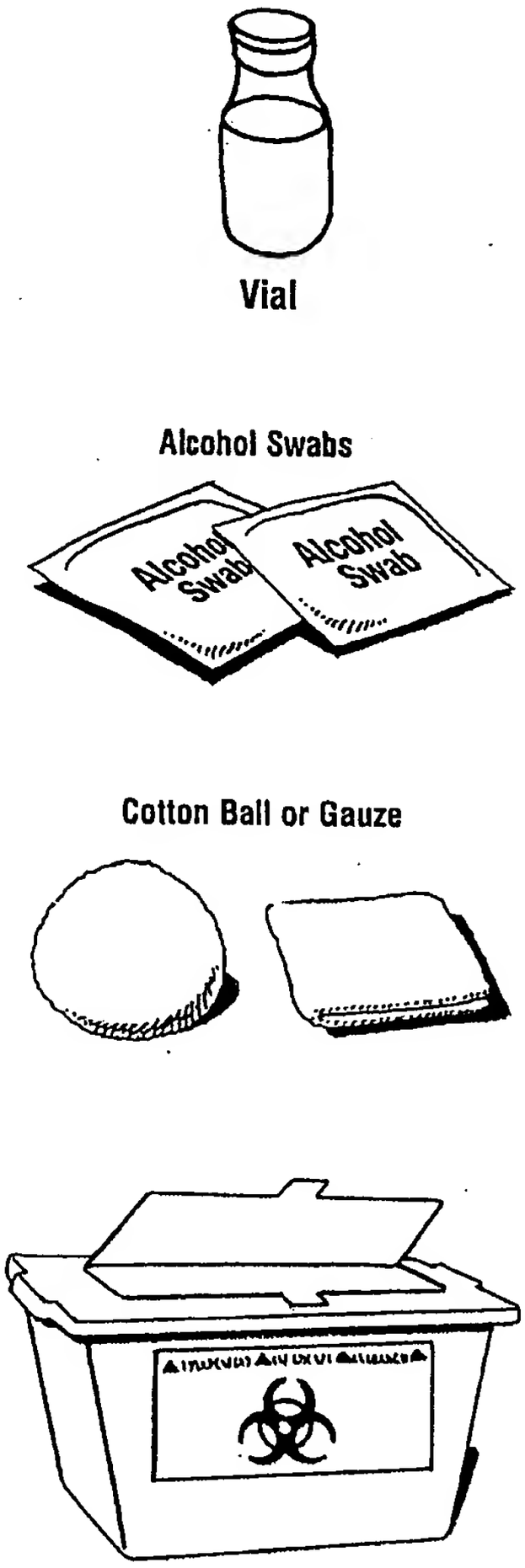


Figure 1.

6. Wash your hands well with soap and warm water before preparing the dose.

9 **PREPARING THE DOSE OF Mircera**

1. Remove the protective colored cap from the vial (see Figure 2).

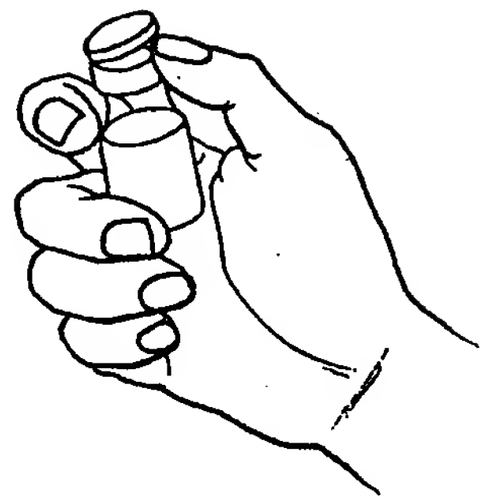


Figure 2.

2. Clean the rubber stopper on the vial with one alcohol swab and put the vial on your flat work surface (see Figure 3).

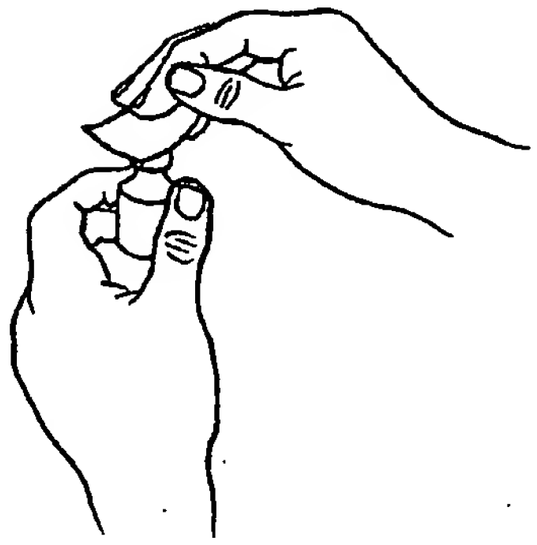


Figure 3.

3. Check the package containing the disposable syringe. If the syringe package is not damaged, open it and take out the syringe. If the package is damaged or has already been opened, do not use that syringe. Throw it away in the puncture-proof disposable container and get a new one.

4. Pull off the needle cover. Then, pull back on the plunger to the dose of Mircera that your healthcare provider has given you (in mL or cc). This will pull air into the syringe (see Figure 4).

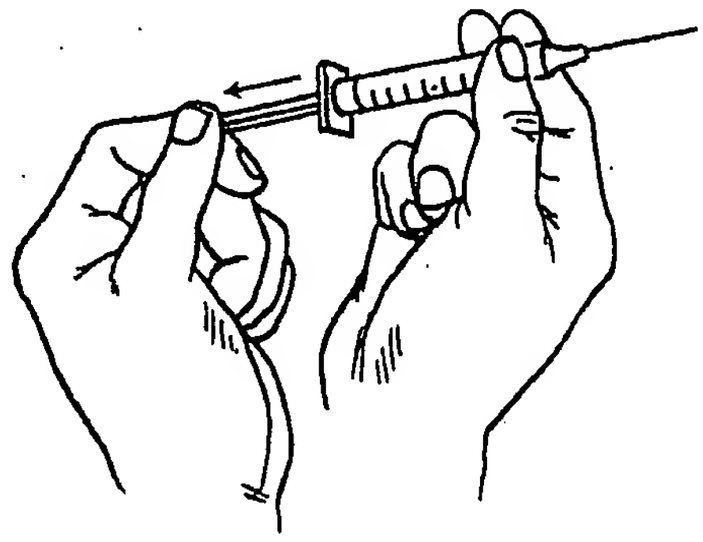


Figure 4.

5. Keep the vial on your flat work surface and insert the needle straight down through the rubber stopper (see Figure 5).

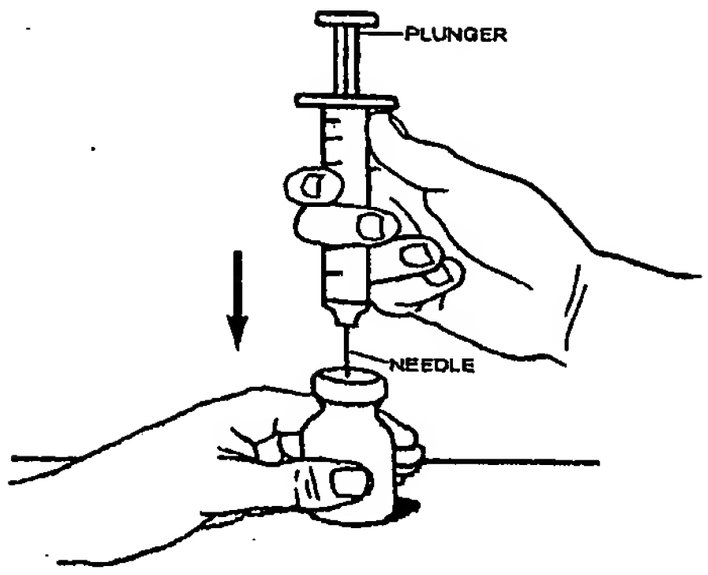


Figure 5.

6. Push down on the plunger of the syringe to inject the air from the syringe into the vial. The air injected into the vial will allow Mircera to be easily withdrawn into the syringe (see Figure 6).

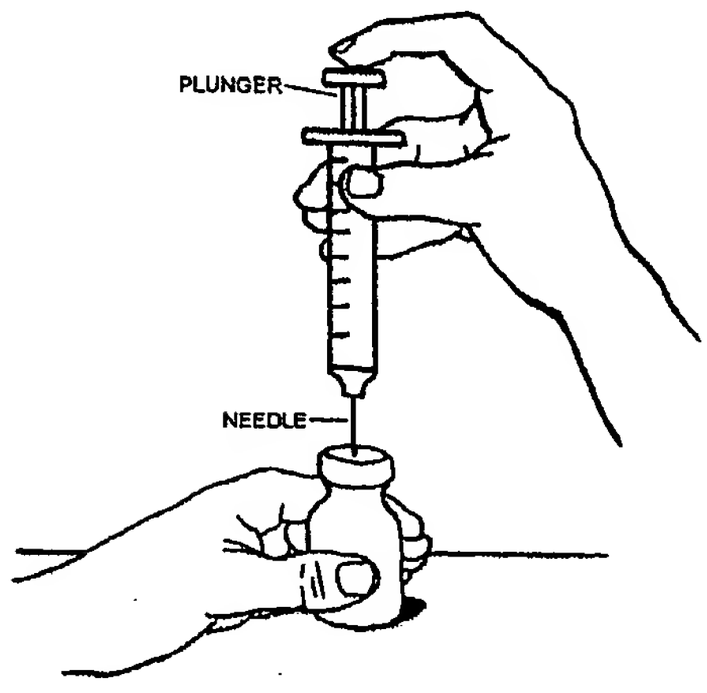


Figure 6.

7. Turn the vial upside down, keeping the needle inside the vial. Make sure that the tip of the needle is in the Mircera liquid. Your other hand will be free to move the plunger. Slowly pull back on the plunger to fill the syringe with Mircera liquid to the number (mL or cc) that matches the dose your healthcare provider has given you (see Figure 7).

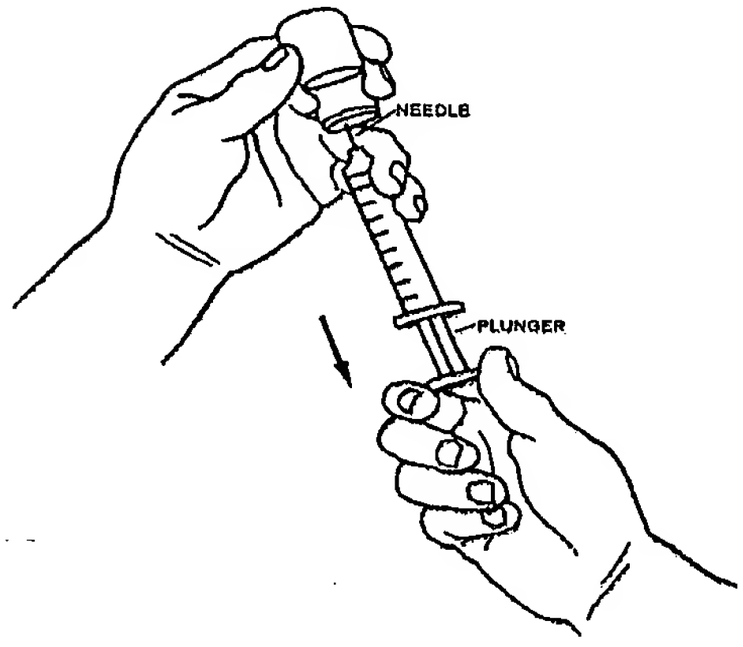


Figure 7.

8. Check for air bubbles in the syringe, keeping the needle inside the vial. Small air bubbles are harmless, but too large an air bubble will not let you draw up the right amount of Mircera. To remove air bubbles, gently tap the syringe with your fingers until the air bubbles rise to the top of the syringe.

9. Slowly push on the plunger to push the solution and the air bubbles out of the syringe and back into the vial (see Figure 8).

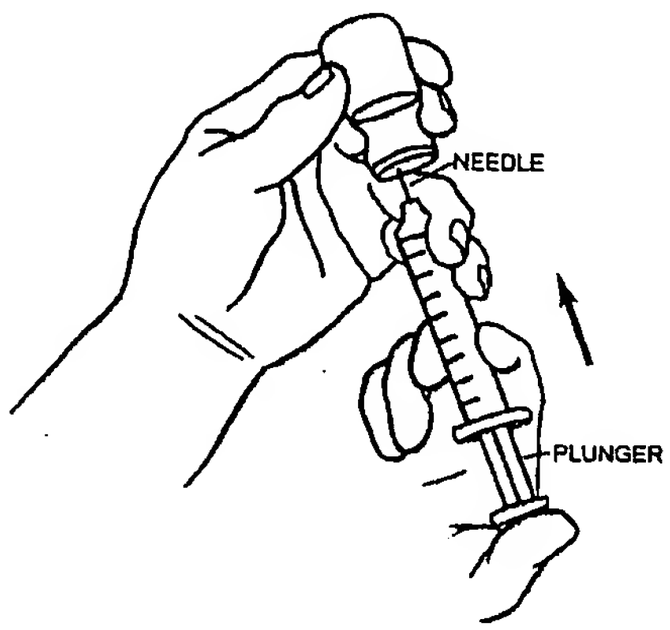


Figure 8.

10. Once again, pull the plunger back to the number on the syringe that matches your dose while making sure the tip of the needle is in the liquid. Check again for air bubbles. If there are still air bubbles, remove them by repeating Steps 8 through 10 (see Figure 9).

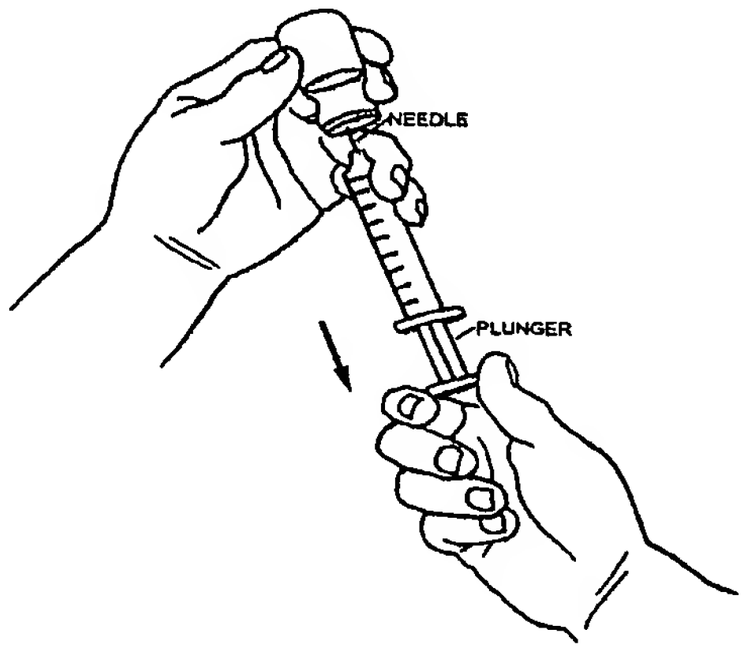


Figure 9.

11. Once you have gotten rid of all air bubbles, check again to make sure you have the right dose. Put the vial on its side with the needle still in it. This will keep the needle from touching anything before you use it (see Figure 10).

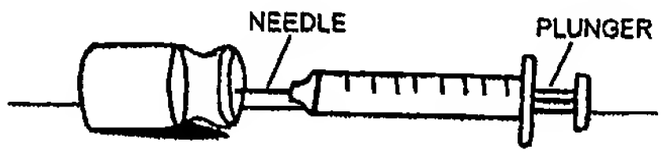


Figure 10.

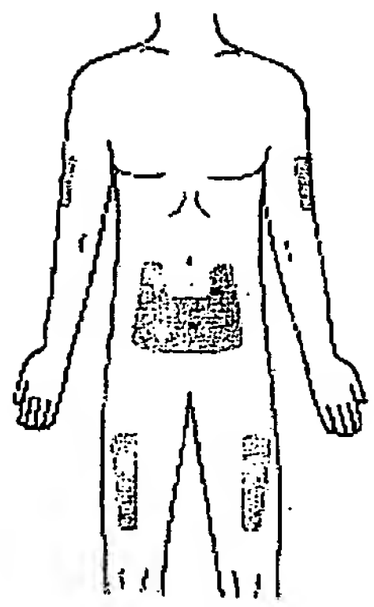
0 **SELECTING AND PREPARING THE INJECTION SITE**

1. Choose an injection site (see Figure 11). The three sites where you can inject Mircera include:

- the outer area of the upper arms
- the front of the middle thighs
- the abdomen (except for the two-inch area around the navel)

Choose a new injection site each time you inject Mircera. This helps to avoid soreness at any one site.

Do not inject Mircera into an area on your body that is tender, red, bruised, hard, or that has scars or stretch marks.



Front

Figure 11.

2. Clean the injection site with a new alcohol swab. Do not touch this area again before giving thc injection (see Figure 12).

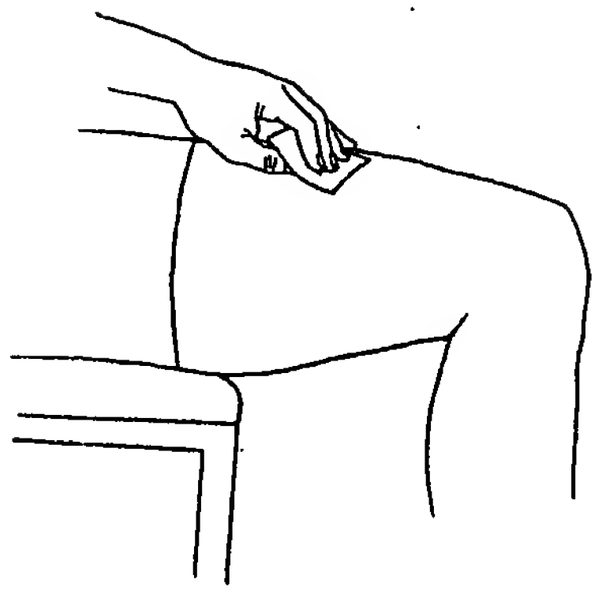


Figure 12.

1 **INJECTING THE DOSE OF Mircera FOR PATIENTS NOT ON HEMODIALYSIS**

1. Hold the syringe in the hand that you will use to inject Mircera. Use the other hand to pinch a fold of skin at the cleaned injection site (see Figure 13).

Figure 13.

2. Hold the syringe like a pencil. Insert the needle in a quick "dart like" motion. Inject either at a slight angle (45 degree angle) or straight up and down (90 degree angle) into the skin (see Figure 14).

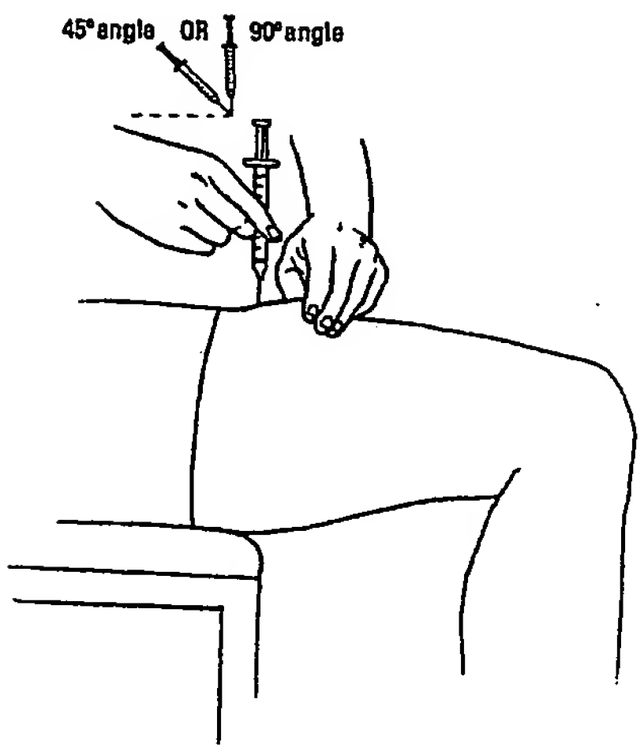


Figure 14.

3. Pull the plunger back slightly after inserting the needle into the skin. If blood comes into the syringe, do not inject Mircera because the needle has entered a blood vessel. Remove the needle from the skin. Slightly reposition the needle within the cleaned area and repeat. If blood does not come, slowly push the plunger all the way down, until all the medicine is injected (see Figure 15).

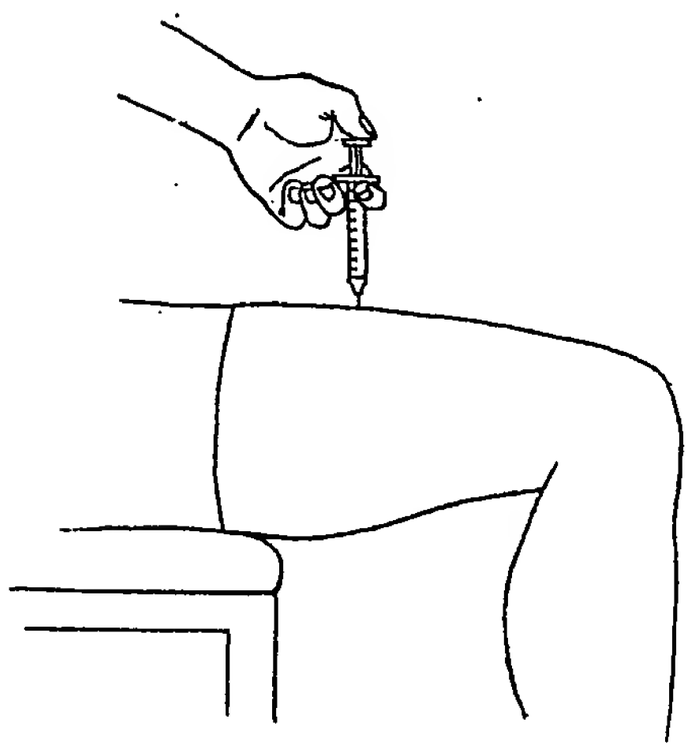


Figure 15.

4. Take the needle out of the skin. Place a cotton ball or gauze over the injection site and press for several seconds (see Figure 16).

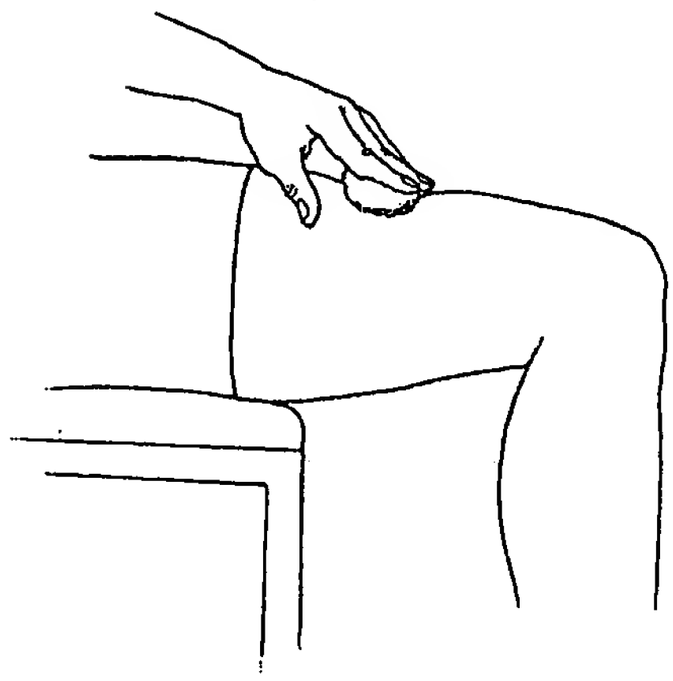


Figure 16.

5. Dispose of the syringe and needle and the vial with any remaining liquid in the puncture-proof disposable container. Use the disposable syringe, needle and vial of medicine one time only.

FOR PATIENTS ON HEMODIALYSIS USING VENOUS INJECTION

1. Clean the venous port of the hemodialysis tubing with a new alcohol swab (see Figure 17).

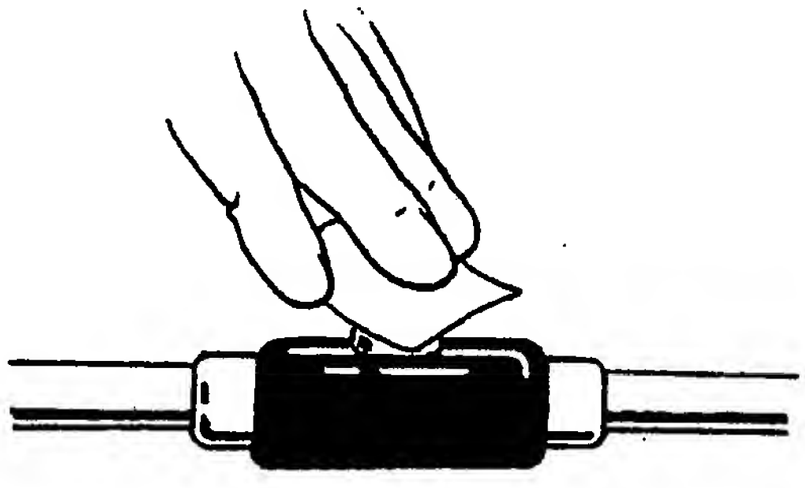


Figure 17.

2. Insert the needle of the syringe into the cleaned venous port and push the plunger all the way down to inject all the medicine (see Figure 18).

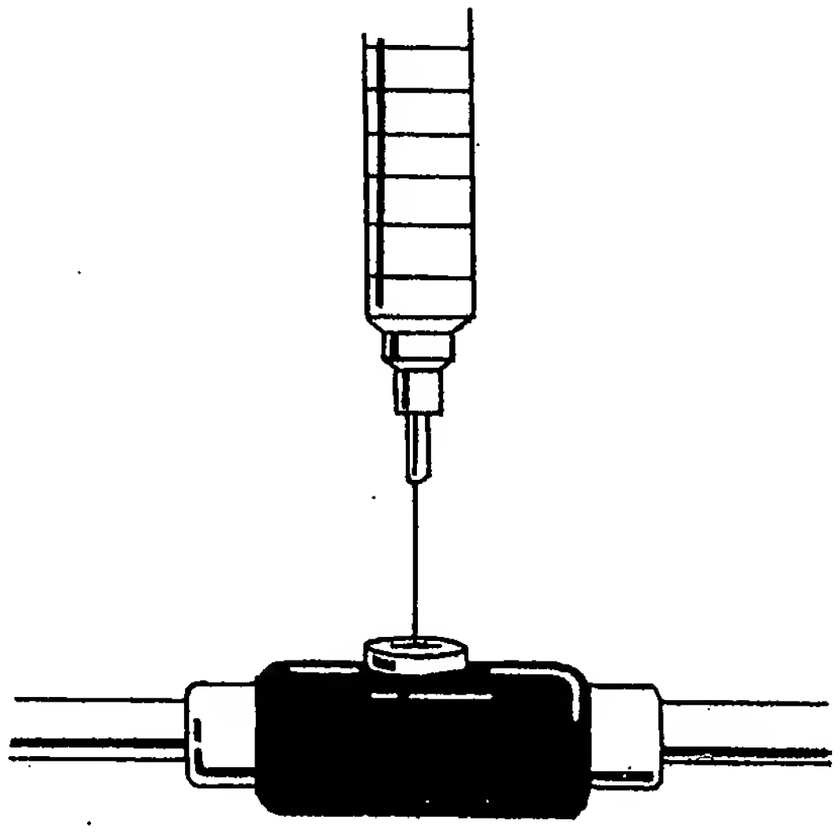


Figure 18.

3. Remove the syringe from the venous port. Dispose of the syringe and needle and the vial with any remaining liquid in the puncture-proof disposable container. Use the disposable syringe, needle and vial of medicine one time only.

Disposing of Syringes and Needles

Follow the required state and local laws for disposal of needles and syringes. Ask your healthcare provider or pharmacist about correct disposal of used syringes and needles.

Use the information below as a general guide:

- Never re-use the needle and syringe
- Place used needle and syringe in the puncture-proof disposable container
- DO NOT use glass or clear plastic containers to throw away the needle and syringe
- Throw away the full puncture-proof disposable container as instructed by your healthcare provider or pharmacist

DO NOT throw away the puncture-proof disposable container in your household trash. DO NOT recycle.
Keep the container out of the reach of children.

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Issued: November 2007

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DEPARTMENT OF HEALTH & HUMAN SERVICES

Public Health Service

Food and Drug Administration
Rockville, MD 20857

Our STN: **BL 125164/0**

NOV 1 4 2007

Hoffman La-Roche
Attention: Krishnan Viswanadhan, Pharm.D.
Associate Director, Drug Regulatory Affairs
340 Kingsland Street
Nutley, NJ 07110

Dear Dr. Viswanadhan:

We have approved your biologics license application (BLA) for methoxy polyethylene glycol-epoetin beta effective this date. You are hereby authorized to introduce or deliver for introduction into interstate commerce, methoxy polyethylene glycol-epoetin beta under your existing Department of Health and Human Services U.S. License No. 0136. Methoxy polyethylene glycol-epoetin beta is indicated for the treatment of anemia associated with chronic renal failure, including patients on dialysis and patients not on dialysis.

Under this license, you are approved to manufacture methoxy polyethylene glycol-epoetin beta drug substance at Roche Diagnostics GmbH in Penzberg, Germany. The final formulated product in vials will be manufactured and filled at Hoffman-La Roche, Ltd., Basel, Switzerland. Labeling and packaging will be performed at Hoffman-La Roche, Ltd., Kaiseraugst, Switzerland. The final formulated product in pre-filled syringes will be manufactured and filled at Roche Diagnostics GmbH, Mannheim, Germany. Labeling and packaging will be performed at Vetter Pharma-Fertigung GmbH & Co. KG, Ravensburg, Germany.

You may label your product with the proprietary name Mircera® and may market it in single-use vials containing 50, 100, 200, 300, 400, 600 or 1000 mcg/mL, and prefilled syringes containing 50, 75, 100, 150, 200 or 250 mcg/0.3 mL, and 400, 600 or 800 mcg/0.6 mL methoxy polyethylene glycol-epoetin beta.

The dating period for methoxy polyethylene glycol-epoetin beta shall be 24 months from the date of manufacture when stored at 2^0 to 8^0C. The data of manufacture shall be defined as the date of final sterile filtration of the formulated drug product. The dating period of your drug substance shall be 36 months when stored at -70^0C to -20^0C. Results of ongoing stability studies should be submitted throughout the dating period, as they become available, including results of stability studies from the first three production lots. The stability protocols in your license application are considered approved for the purpose of extending the expiration dating period of your drug substance and drug product under 21 CFR 601.12.

You currently are not required to submit samples of future lots of methoxypolyethylene glycol-epoetin beta to the Center for Drug Evaluation and Research (CDER) for release by the Director, CDER, under 21 CFR 610.2. We will continue to monitor compliance with 21 CFR 610.1 requiring completion of tests for conformity with standards applicable to each product prior to release of each lot.

You must submit information to your biologics license application for our review and written approval under 21 CFR 601.12 for any changes in the manufacturing, testing, packaging or labeling of methoxy polyethylene glycol-epoetin beta, or in the manufacturing facilities.

Your application was not referred to an FDA advisory committee for review for the following reasons: The safety considerations for approved Erythropoiesis-Stimulating Agents (ESAs) were discussed at a joint meeting of the Cardiovascular and Renal Drugs Advisory Committee and the Drug Safety and Risk Management Advisory Committee on September 11, 2007. The committees provided recommendations regarding the labeling for all ESA products and these recommendations were incorporated into the previously approved ESA labels, as well as the label for your product. Furthermore, clinical studies of your product were similar in design to previously approved products in the ESA class and your product's efficacy did not pose unique concerns in the indicated patient population, beyond the issues generally applicable to ESAs. In addition, evaluation of your product's safety when used in the treatment of the anemia due to chronic renal failure did not reveal particular safety issues that were unexpected for a member of the ESA class, when used for this specific indication.

All applications for new active ingredients, new dosage forms, new indications, new routes of administration, and new dosing regimens are required to contain an assessment of the safety and effectiveness of the product in pediatric patients unless this requirement is waived or deferred. We are waiving the pediatric study requirement for neonates through 4 years and deferring submission of your pediatric studies for ages 5 to 17 years until December 2012.

We acknowledge your written commitments as described in your letters of August 13, 2007, October 16, 2007, and November 8, 2007 as outlined below:

Postmarketing Study Commitments subject to reporting requirements of 21 CFR 601.70.

Your deferred pediatric studies required under the Pediatric Research Equity Act (PREA) are considered required postmarketing study commitments. The statuses of these postmarketing studies shall be reported annually according to 21 CFR 601.70. These commitments are listed below.

1. To conduct a multi-center, dose-finding study to determine the optimum starting dose of intravenously administered methoxy polyethylene glycol-epoetin beta when used for the maintenance treatment of anemia in pediatric patients ages 5 to 17 years who have chronic kidney disease and are undergoing dialysis.
   The protocol for this study has been submitted.
   Patient enrollment will begin by July 31, 2008.
   The final study report will be submitted by October 30, 2009.

2. To conduct a multi-center, randomized, controlled, parallel-group study to confirm the optimal methoxy polyethylene glycol-epoetin beta dosage when used for the maintenance treatment of anemia in pediatric patients ages 5 to 17 years who have chronic kidney disease, inclusive of patients undergoing dialysis as well as patients who are not undergoing dialysis.
The protocol for this study has been submitted.
Patient enrollment will begin by May 31, 2010.
The final study report will be submitted by April 30, 2012.

Submit final study reports to this BLA. For administrative purposes, all submissions related to these pediatric postmarketing study commitments must be clearly designated **"Required Pediatric Study Commitments".**

3. To conduct a phase 4, randomized, controlled, open label, multicenter parallel group study to assess the all cause mortality and cardiovascular morbidity in anemic patients with chronic kidney disease who are on dialysis and not undergoing dialysis. The study will enroll patients with a broad range of C-reactive protein blood concentrations and will randomize patients to treatment with either methoxy polyethylene glycol-epoetin beta or another erythropoiesis-stimulating agent.
The final study protocol will be submitted by February 29, 2008.
Patient enrollment will begin by July 31, 2008.
The final study report will be submitted by September 29, 2012.

Postmarketing Study Commitments not subject to reporting requirements of 21 CFR 601.70.

4. To provide comprehensive assay validation package for the neutralizing antibody assays. In addition to standard validation parameters the validation submission will include:

   a. The rationale and supporting data for the following:

      - proposed criteria that designate samples as positive or negative in the neutralizing assay.
      - the system suitability criteria.

   b. A description of the negative control that will be used in routine running of the assay as well as supporting qualification data.

   c. The assay standard operating procedure (SOP) document.

   The assay validation package will be submitted as a supplement to this BLA, by June 30, 2008.

We request that you submit clinical protocols to your IND, with a cross-reference letter to this BLA, STN BL 125164. Submit nonclinical and chemistry, manufacturing, and controls protocols and all study final reports to your BLA, STN BL 125164. Please use the following designators to label prominently all submissions, including supplements, relating to these postmarketing study commitments as appropriate:

- Postmarketing Study Commitment Protocol
- Postmarketing Study Commitment - Final Study Report
- Postmarketing Study Correspondence
- Annual Status Report of Postmarketing Study Commitments.

For each postmarketing study subject to the reporting requirements of 21 CFR 601.70, you must describe the status in an annual report on postmarketing studies for this product. The status report for each study should include:

- information to identify and describe the postmarketing commitment,
- the original schedule for the commitment,
- the status of the commitment (i.e. pending, ongoing, delayed, terminated, or submitted),
- an explanation of the status including, for clinical studies, the patient accrual rate (i.e. number enrolled to date and the total planned enrollment), and
- a revised schedule if the study schedule has changed and an explanation of the basis for the revision.

As described in 21 CFR 601.70(e), we may publicly disclose information regarding these postmarketing studies on our Web site (http://www.fda.gov/cder/pmc/default.htm). Please refer to the February 2006 Guidance for Industry: Reports on the Status of Postmarketing Study Commitments - Implementation of Section 130 of the Food and Drug Administration Modernization Act of 1997 (see http://www.fda.gov/cder/guidance/5569fnl.htm) for further information.

Under 21 CFR Part 208, we have determined that this product poses a serious and significant public health concern requiring the distribution of a Medication Guide. Methoxy polyethylene glycol-epoetin beta is a product for which patient labeling could help prevent serious adverse effects and inform the patient of serious risks relative to benefit that could affect their decisions to use, or continue to use, the product. Therefore, a Medication Guide is necessary for safe and effective use of this product and FDA hereby approves the draft Medication Guide you submitted on November 9, 2007. Please note that:

- this Medication Guide must be reprinted immediately following the last section of labeling or, alternatively, accompany the prescription drug labeling [21 CFR 201.57(c)(18) or 21 CFR 201.80(f)(2)];
- you are responsible for ensuring that this Medication Guide is available for distribution to every patient who is dispensed a prescription for this product [21 CFR 208.24];
- the final printed Medication Guide distributed to patients must conform to all conditions described in 21 CFR 208.20, including a minimum of 10 point text; and

- you are responsible for ensuring that the label of each container or package includes a prominent and conspicuous instruction to authorized dispensers to provide a Medication Guide to each patient to whom the drug is dispensed, and states how the Medication Guide is provided [21 CFR 208.24(d)].

You must submit adverse experience reports under the adverse experience reporting requirements for licensed biological products (21 CFR 600.80). You should submit postmarketing adverse experience reports to the Central Document Room, Center for Drug Evaluation and Research, Food and Drug Administration, 5901-B Ammendale Road, Beltsville, MD 20705-1266. Prominently identify all adverse experience reports as described in 21 CFR 600.80.

The MedWatch-to-Manufacturer Program provides manufacturers with copies of serious adverse event reports that are received directly by the FDA. New molecular entities and important new biologics qualify for inclusion for three years after approval. Your firm is eligible to receive copies of reports for this product. To participate in the program, please see the enrollment instructions and program description details at www.fda.gov/medwatch/report/mmp.htm.

You must submit distribution reports under the distribution reporting requirements for licensed biological products (21 CFR 600.81).

You must submit reports of biological product deviations under 21 CFR 600.14. You should promptly identify and investigate all manufacturing deviations, including those associated with processing, testing, packing, labeling, storage, holding and distribution. If the deviation involves a distributed product, may affect the safety, purity, or potency of the product, and meets the other criteria in the regulation, you must submit a report on Form FDA-3486 to the Division of Compliance Risk Management and Surveillance (HFD-330), Center for Drug Evaluation and Research, Food and Drug Administration, 5600 Fishers Lane, Rockville, MD 20857. Biological product deviations sent by courier or overnight mail should be addressed to Food and Drug Administration, CDER, Office of Compliance, Division of Compliance Risk Management and Surveillance, HFD-330, Montrose Metro 2, 11919 Rockville Pike, Rockville, MD 20852.

Within 14 days of the date of this letter, submit content of labeling [21 CFR 601.14(b)] in structured product labeling (SPL) format, as described at http://www.fda.gov/oc/datacouncil/spl.html, that is identical in content to the enclosed labeling text. Upon receipt, we will transmit that version to the National Library of Medicine for public dissemination. For administrative purposes, please designate this submission "Product Correspondence – Final SPL for approved STN BL 125164/0."

Marketing the product with labeling that is not identical to the approved labeling text may render the product misbranded and an unapproved new drug.

You may submit draft copies of the proposed introductory advertising and promotional labeling with a cover letter requesting advisory comments to the Food and Drug Administration, Center for Drug Evaluation and Research, Division of Drug Marketing, Advertising and Communication, 5901-B Ammendale Road, Beltsville, MD 20705-1266. Final printed advertising and promotional labeling should be submitted at the time of initial dissemination, accompanied by a FDA Form 2253.

All promotional claims must be consistent with and not contrary to approved labeling. You should not make a comparative promotional claim or claim of superiority over other products unless you have substantial evidence to support that claim.

Please refer to http://www.fda.gov/cder/biologics/default.htm for information regarding therapeutic biological products, including the addresses for submissions.

Sincerely,

Richard Pazdur, M.D.

Richard Pazdur, M.D.
Director
Office of Oncology Drug Product
Center for Drug Evaluation and Research

Attachment: Physician Label
Medication Guide
Patient Instructions for Use for Vials
Patient Instructions for Use for Pre-filled Syringes

C

US006583272B1

(12) **United States Patent**
Bailon

(10) **Patent No.: US 6,583,272 B1**
(45) **Date of Patent: Jun. 24, 2003**

(54) **ERYTHROPOIETIN CONJUGATES**

(75) Inventor: **Pascal Sebastian Bailon**, Florham Park, NJ (US)

(73) Assignee: **Hoffmann-La Roche Inc.**, Nutley, NJ (US)

(*) Notice: Subject to any disclaimer, the term of this patent is extended or adjusted under 35 U.S.C. 154(b) by 60 days.

(21) Appl. No.: **09/604,938**

(22) Filed: **Jun. 27, 2000**

Related U.S. Application Data

(60) Provisional application No. 60/142,254, filed on Jul. 2, 1999, provisional application No. 60/150,225, filed on Aug. 23, 1999, provisional application No. 60/151,548, filed on Aug. 31, 1999, and provisional application No. 60/166,151, filed on Nov. 17, 1999.

(51) **Int. Cl.**[7] **C07K 14/505**; C07K 17/00; A61K 38/17; A61K 39/00

(52) **U.S. Cl.** **530/397**; 530/350; 514/2; 514/8; 424/194.1; 424/195.11

(58) **Field of Search** 514/2, 8; 530/350, 530/397; 424/195.11, 194.1

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* cited by examiner

Primary Examiner—Gary Kunz
Assistant Examiner—Regina M. DeBerry
(74) *Attorney, Agent, or Firm*—George W. Johnston; Patricia S. Rocha-Tramaloni

(57) **ABSTRACT**

Conjugates of erythropoietin with poly(ethylene glycol) comprise an erythropoietin glycoprotein having at least one free amino group and having the in vivo biological activity of causing bone marrow cells to increase production of reticulocytes and red blood cells and selected from the group consisting of human erythropoietin and analogs thereof which have sequence of human erythropoietin modified by the addition of from 1 to 6 glycosylation sites or a rearrangement of at least one glycosylation site; the glycoprotein being covalently linked to "n" poly(ethylene glycol) groups of the formula —CO—$(CH_2)_x(OCH_2CH_2)_m$—OR with the carbonyl of each poly(ethylene glycol) group forming an amide bond with one of said amino groups; wherein R is lower alkyl; x is 2 or 3; m is about 450 to about 900; n is from 1 to 3; and n and m are chosen so that the molecular weight of the conjugate minus the erythropoietin glycoprotein is from 20 kilodaltons to 100 kilodaltons.

15 Claims, 3 Drawing Sheets

Figure 1

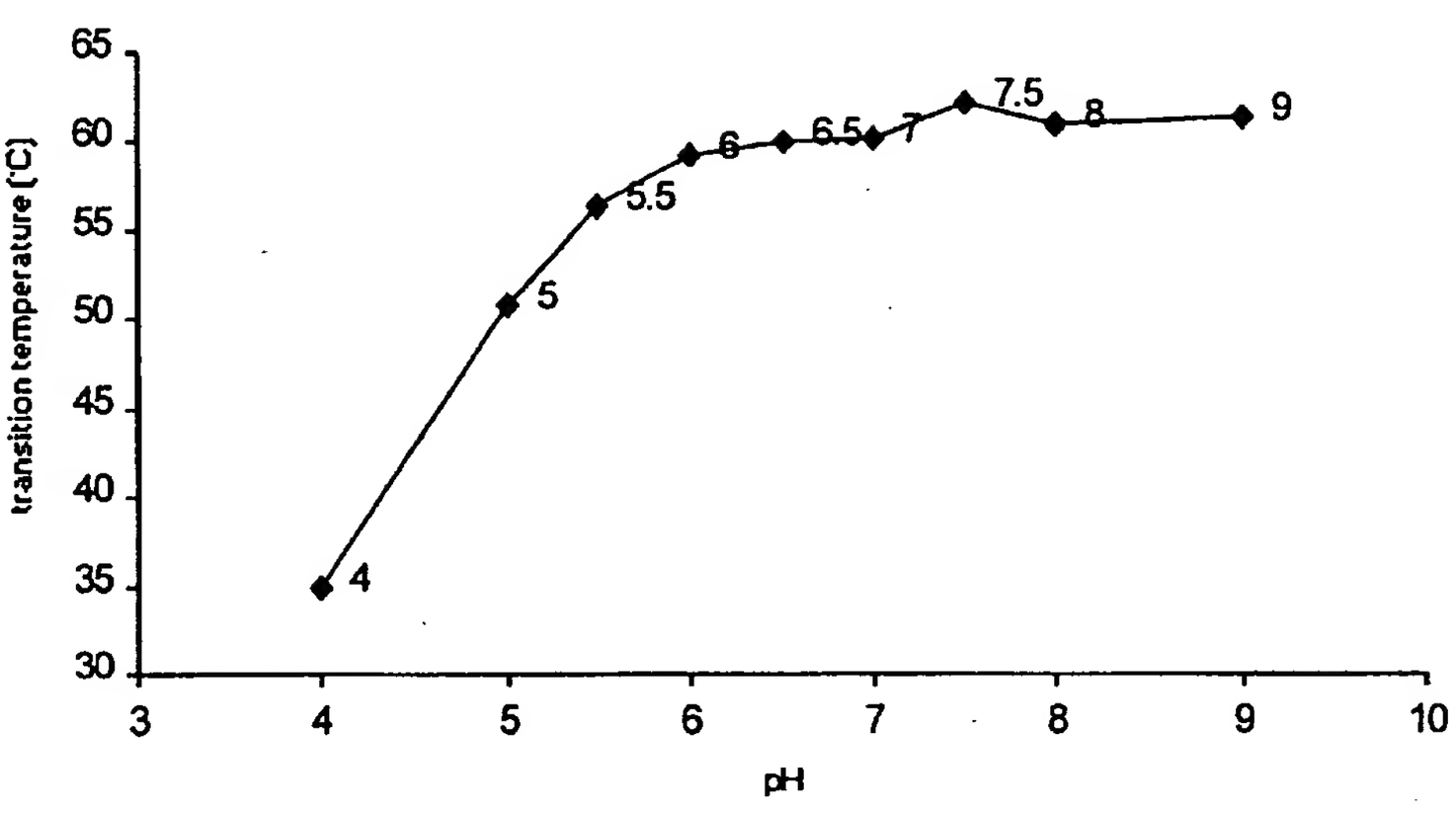


Figure 2

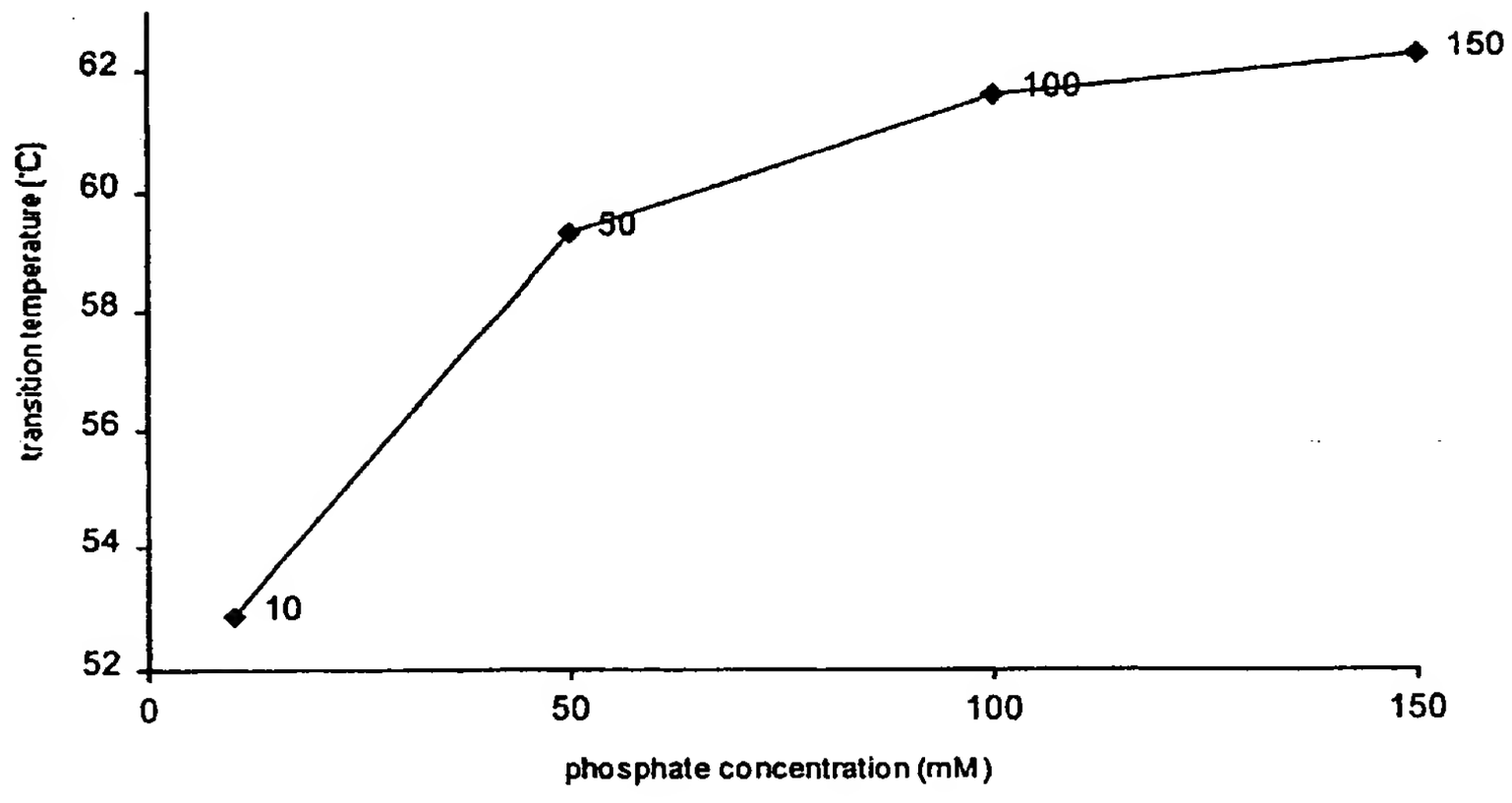


Figure 3

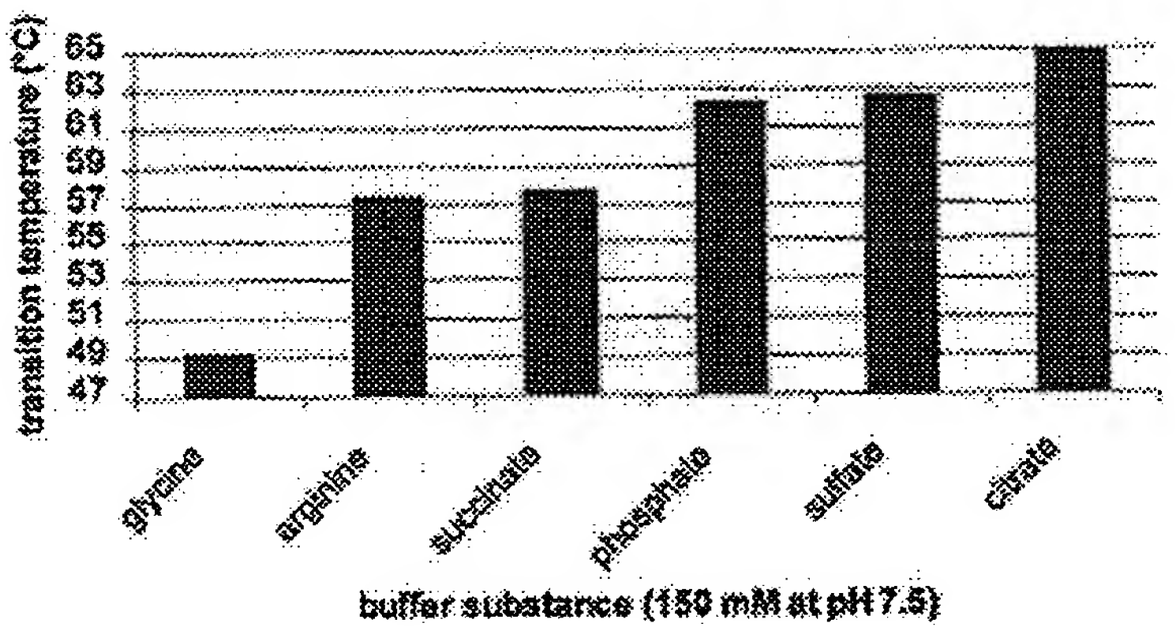


Figure 4

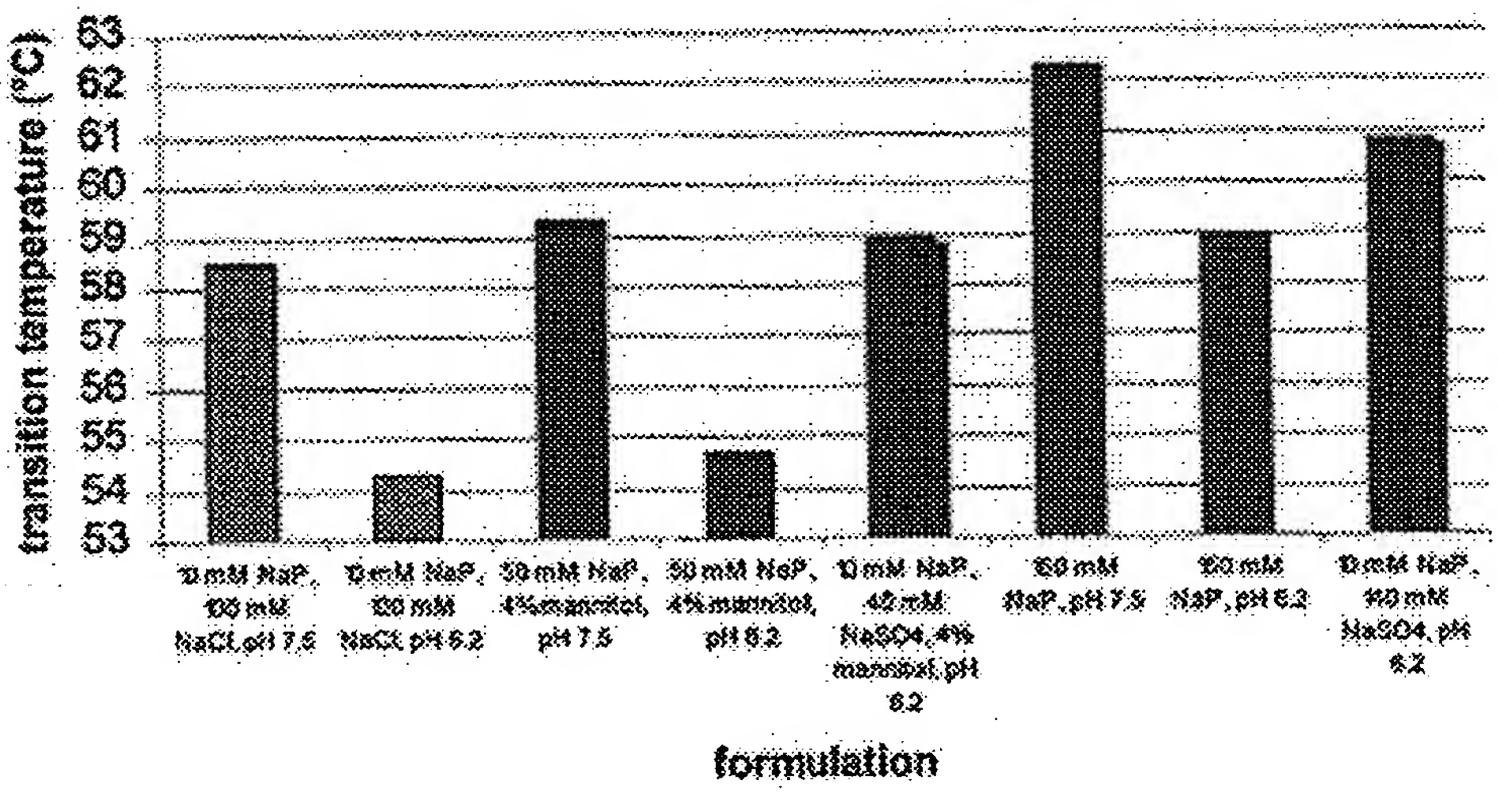


Figure 5

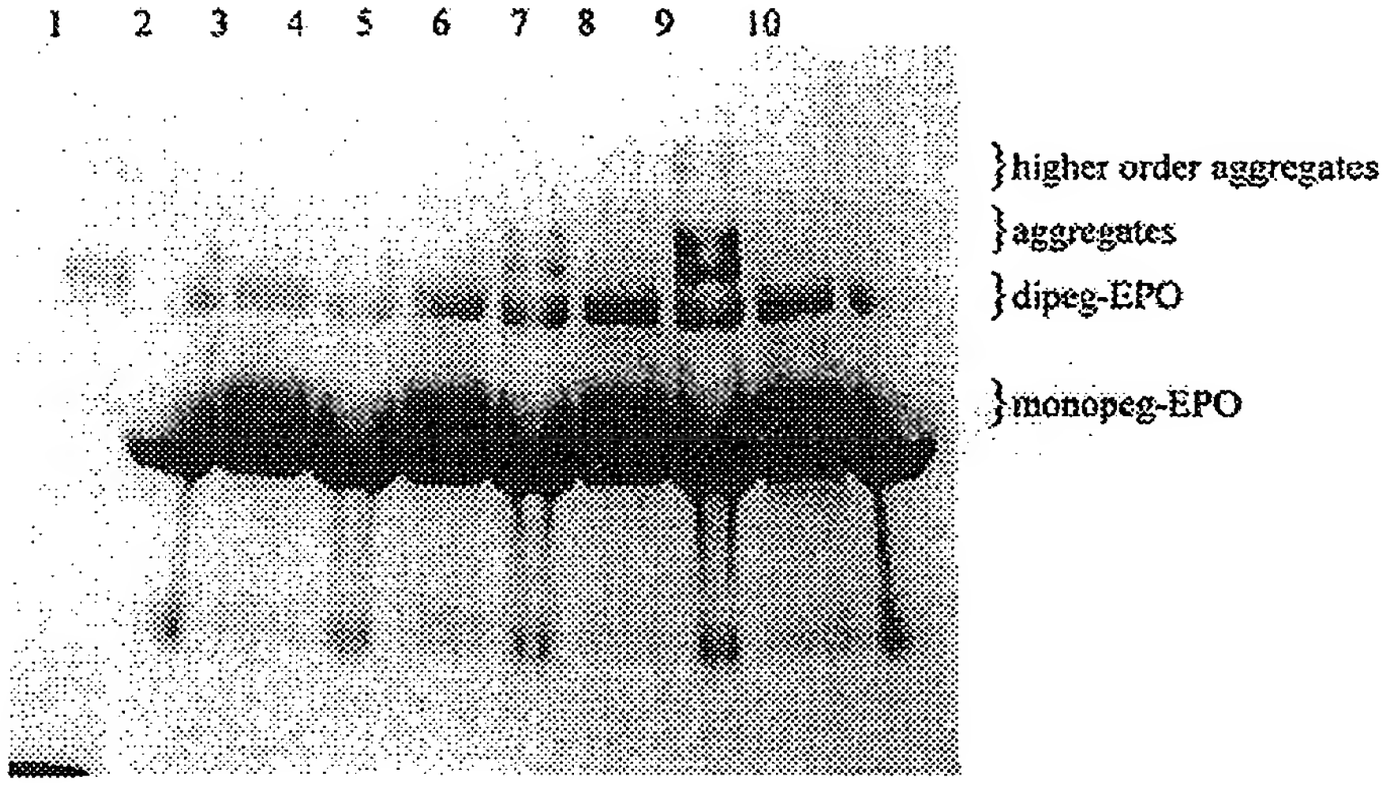
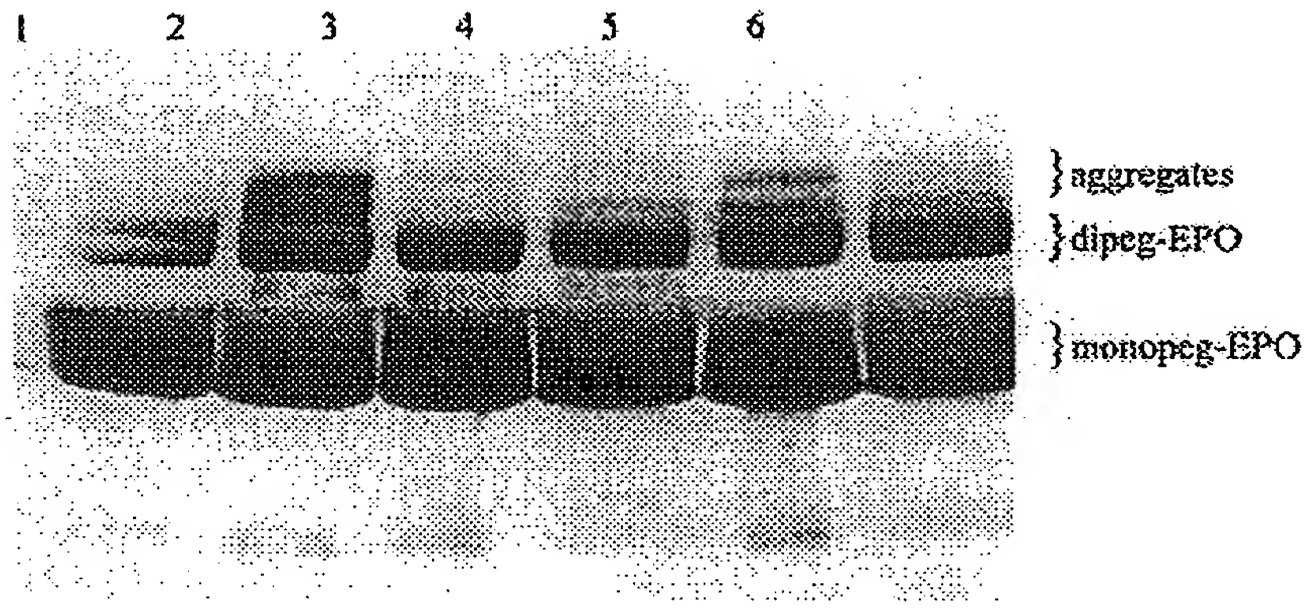


Figure 6



ERYTHROPOIETIN CONJUGATES

CROSS-REFERENCE TO RELATED APPLICATIONS

The priority of U.S. Provisional Application No. 60/142,254, filed Jul. 2, 1999; No. 60/150,225, filed Aug. 23, 1999; No. 60/151,548, filed Aug. 31, 1999; and No. 60/166,151, filed Nov. 17, 1999 is claimed.

BACKGROUND OF THE INVENTION

Erythropoiesis is the production of red blood cells, which occurs to offset cell destruction. Erythropoiesis is a controlled physiological mechanism that enables sufficient red blood cells to be available for proper tissue oxygenation. Naturally occurring human erythropoietin (hEPO) is produced in the kidney and is the humoral plasma factor which stimulates red blood cell production (Carnot, P and Deflandre, C (1906) C. R. Acad. Sci. 143: 432; Erslev, A J (1953 Blood 8: 349; Reissmann, K R (1950) Blood 5: 372; Jacobson, L O, Goldwasser, E, Freid, W and Plzak, L F (1957) Nature 179: 6331–4). Naturally occurring EPO stimulates the division and differentiation of committed erythroid progenitors in the bone marrow and exerts its biological activity by binding to receptors on erythroid precursors (Krantz, B S (1991) Blood 77: 419).

Erythropoietin has been manufactured biosynthetically using recombinant DNA technology (Egrie, J C, Strickland, T W, Lane, J et al. (1986) Immunobiol. 72: 213–224) and is the product of a cloned human EPO gene inserted into and expressed in the ovarian tissue cells of the chinese hamster (CHO cells). The primary structure of the predominant, fully processed form of hEPO is illustrated in SEQ ID NO:1. There are two disulfide bridges between Cys^{7}-Cys^{161} and Cys^{29}–Cys^{33}. The molecular weight of the polypeptide chain of EPO without the sugar moieties is 18,236 Da. In the intact EPO molecule, approximately 40% of the molecular weight are accounted for by the carbohydrate groups that glycosylate the protein at glycosylation sites on the protein (Sasaki, H, Bothner, B, Dell, A and Fukuda, M (1987) J. Biol. Chem. 262: 12059).

Because human erythropoietin is essential in red blood cell formation, the hormone is useful in the treatment of blood disorders characterized by low or defective red blood cell production. Clinically, EPO is used in the treatment of anemia in chronic renal failure patients (CRF) (Eschbach, J W, Egri, J C, Downing, M R et al. (1987) NEJM 316: 73–78; Eschbach, J W, Abdulhadi, M H, Browne, J K et al. (1989) Ann. Intern. Med. 111: 992; Egrie, J C, Eschbach, J W, McGuire, T, Adamson, J W (1988) Kidney Intl. 33: 262; Lim, V S, Degowin, R L, Zavala, D et al. (1989) Ann. Intern. Med. 110: 108–114) and in AIDS and cancer patients undergoing chemotherapy (Danna, R P, Rudnick, S A, Abels, R I In: M B, Garnick, ed. Erythropoietin in Clinical Applications—An International Perspective. New York, N.Y.: Marcel Dekker; 1990: p. 301–324). However, the bioavailability of commercially available protein therapeutics such as EPO is limited by their short plasma half-life and susceptibility to protease degradation. These shortcomings prevent them from attaining maximum clinical potency.

SUMMARY OF THE INVENTION

This invention provides an erythropoietin conjugate, said conjugate comprising an erythropoietin glycoprotein having at least one free amino group and having the in vivo biological activity of causing bone marrow cells to increase production of reticulocytes and red blood cells and selected from the group consisting of human erythropoietin and analogs thereof which have sequence of human erythropoietin modified by the addition of from 1 to 6 glycosylation sites or a rearrangement of at least one glycosylation site; said glycoprotein being covalently linked to "n" poly (ethylene glycol) groups of the formula —CO—$(CH_2)_x$—$OCH_2CH_2)_m$—OR with the —CO (i.e. carbonyl) of each poly(ethylene glycol) group forming an amide bond with one of said amino groups; wherein R is lower alkyl; x is 2 or 3; m is from about 450 to about 900; n is from 1 to 3; and n and m are chosen so that the molecular weight of the conjugate minus the erythropoietin glycoprotein is from 20 kilodaltons to 100 kilodaltons. This invention further provides compositions containing conjugates described herein in which the percentage of conjugates in the composition in which n is 1 is at least ninety percent.

Compared to unmodified EPO (i.e., EPO without a PEG attached) and conventional PEG-EPO conjugates, the present conjugates have an increased circulating half-life and plasma residence time, decreased clearance, and increased clinical activity in vivo. The conjugates of this invention have the same uses as EPO. In particular, the conjugates of this invention are useful to treat patients by stimulating the division and differentiation of committed erythroid progenitors in the bone marrow in the same way EPO is used to treat patients.

BRIEF DESCRIPTION OF THE FIGURES

FIG. 1: Influence of pH on thermal stability. The transition temperature is plotted against the pH.

FIG. 2: Influence of ionic strength on thermal stability. The transition temperature is plotted against the phosphate concentration.

FIG. 3: Dependence of thermal stability on buffer substance.

FIG. 4 shows that sulfate is also a suitable buffer/additive at low pH (e.g. pH 6.2), whereas phosphate is less suitable at pH 6.2 compared to pH 7.5. This shows that sulfate keeps the thermal stability high, even at low pH.

FIG. 5: Dependency of peg-EPO aggregation on pH. Peg-EPO samples after heat stress (as described above) were analyzed by SDS-PAGE. Proteins were stained with silver. Lane 1: molecular weight standard. Lane 2: pH 5. Lane 3: pH 5, reduced. Lane 4: pH 6. Lane 5: pH 6, reduced. Lane 6: pH 6.5. Lane 7: pH 6.5, reduced. Lane 8: pH 7. Lane 9: pH 7, reduced. Lane 10: peg-EPO, unstressed.

FIG. 6 shows that the use of 1 mg/ml acetylcysteine as an antioxidant prevents the formation of aggregates under heat stress.

DETAILED DESCRIPTION OF THE INVENTION

This invention provides conjugates, said conjugates comprising an erythropoietin glycoprotein having at least one free amino group and having the in vivo biological activity of causing bone marrow cells to increase production of reticulocytes and red blood cells and selected from the group consisting of human erythropoietin and analogs thereof which have sequence of human erythropoietin modified by the addition of from 1 to 6 glycosylation sites or a rearrangement of at least one glycosylation site; said glycoprotein being covalently linked to "n" poly(ethylene glycol) groups of the formula —CO—$CH_2)_x$—$(OCH_2CH_2)_m$—OR with the —CO (i.e. carbonyl) of each poly(ethylene glycol)

group forming an amide bond with one of said amino groups; wherein R is lower alkyl; x is 2 or 3; m is from about 450 to about 900; n is from 1 to 3; and n and m are chosen so that the molecular weight of the conjugate minus the erythropoietin glycoprotein is from 20 kilodaltons to 100 kilodaltons.

It has been found that the conjugates of this invention can be used in the same manner as unmodified EPO. However, the conjugates of this invention have an increased circulating half-life and plasma residence time, decreased clearance, and increased clinical activity in vivo. Because of these improved properties, the conjugates of this invention can be administered once weekly instead of the three times weekly for unmodified EPO. Decreased frequency of administration is expected to result in improved patient compliance leading to improved treatment outcomes, as well as improved patient quality of life. Compared to conventional conjugates of EPO linked to poly(ethylene glycol) it has been found that conjugates having the molecular weight and linker structure of the conjugates of this invention have an improved potency, stability, AUC, circulating half-life, and cost of goods profile.

The conjugates in accordance of this invention can be administered in a therapeutically effective amount to patients in the same way EPO is administered. The therapeutically effective amount is that amount of conjugate necessary for the in vivo biological activity of causing bone marrow cells to increase production of reticulocytes and red blood cells. The exact amount of conjugate is a matter of preference subject to such factors as the exact type of condition being treated, the condition of the patient being treated, as well as the other ingredients in the composition. The pharmaceutical compositions containing the conjugate may be formulated at a strength effective for administration by various means to a human patient experiencing blood disorders characterized by low or defective red blood cell production. Average therapeutically effective amounts of the conjugate may vary and in particular should be based upon the recommendations and prescription of a qualified physician.

The erythropoietin glycoprotein products prepared in accordance with this invention may be prepared in pharmaceutical compositions suitable for injection with a pharmaceutically acceptable carrier or vehicle by methods known in the art. Among the preferred pharmaceutically acceptable carriers for formulating the products of the invention are human serum albumn, human plasma proteins, etc.

The term "erythropoietin" or "EPO" refers to a glycoprotein, having the amino acid sequence set out in (SEQ ID NO: 1) or (SEQ ID NO: 2) or an amino acid sequence substantially homologous thereto, whose biological properties relate to the stimulation of red blood cell production and the stimulation of the division and differentiation of committed erythroid progenitors in the bone marrow. As used herein, these terms include such proteins modified deliberately, as for example, by site directed mutagenesis or accidentally through mutations. These terms also include analogs having from 1 to 6 additional sites for glycosylation, analogs having at least one additional amino acid at the carboxy terminal end of the glycoprotein, wherein the additional amino acid includes at least one glycosylation site, and analogs having an amino acid sequence which includes a rearrangement of at least one site for glycosylation. These terms include both natural and recombinantly produced human erythropoietin. The erythropoietin conjugates of this invention can be represented by Formula 1:

$$P\text{—}[NHCO\text{—}CH_2)_x\text{—}OCH_2CH_2)_m\text{—}OR]_n \quad (I)$$

wherein x, m, n and R are as above. In Formula I, P is the residue of an erythropoietin glycoprotein described herein, (i.e. without the amino group or amino groups which form an amide linkage with the carbonyl shown in Formula I), having the in vivo biological activity of causing bone marrow cells to increase production of reticulocytes and red blood cells. P may be selected from the group consisting of residues of human erythropoietin and analogs thereof having from 1 to 6 additional sites for glycosylation. As set out in detail below, the preparation and purification of EPO are well known in the art. By EPO is meant the natural or recombinant protein, preferably human, as obtained from any conventional source such as tissues, protein synthesis, cell culture with natural or recombinant cells. Any protein having the. activity of EPO, such as muteins or otherwise modified proteins, is encompassed. Recombinant EPO may be prepared via expression in CHO—, BHK— or HeLa cell lines, by recombinant DNA technology or by endogenous gene activation. Expression of proteins, including EPO, by endogenous gene activation is well known in the art and is disclosed, for example in U.S. Pat. Nos. 5,733,761, 5,641,670, and 5,733,746, and international patent publication Nos. WO 93/09222, WO 94/12650, WO 95/31560, WO 90/11354, WO 91/06667 and WO 91/09955, the contents of each of which are incorporated herein by reference. The preferred EPO species for the preparation of erythropoietin glycoprotein products are human EPO species. More preferably, the EPO species is the human EPO having the amino acid sequence set out in SEQ ID NO:1 or SEQ ID NO:2, more preferably the amino acid sequence SEQ ID NO:1.

In an embodiment, P may be the residue of a glycoprotein analog having from 1 to 6 additional sites for glycosylation. Glycosylation of a protein, with one or more oligosaccharide groups, occurs at specific locations along a polypeptide backbone and greatly affects the physical properties of the protein such as protein stability, secretion, subcellular localization, and biological activity. Glycosylation is usually of two types. O-linked oligosaccharides are attached to serine or threonine residues and N-linked oligosaccharides are attached to asparagine residues. One type of oligosaccharide found on both N-linked and O-linked oligosaccharides is N-acetylneuraminic acid (sialic acid), which is a family of amino sugars containing 9 or more carbon atoms. Sialic acid is usually the terminal residue on both N-linked and O-linked oligosaccharides and, because it bears a negative charge, confers acidic properties to the glycoprotein. Human erythropoietin, having 165 amino acids, contains three N-linked and one O-linked oligosaccharide chains which comprise about 40% of the total molecular weight of the glycoprotein. N-linked glycosylation occurs at asparagine residues located at positions 24, 38, and 83 and O-linked glycosylation occurs at a serine residue located at position 126. The oligosaccharide chains are modified with terminal sialic acid residues. Enzymatic removal of all sialic acid residues from the glycosylated erythropoietin results in loss of in vivo activity but not in vitro activity because sialylation of erythropoietin prevents its binding, and subsequent clearance, by hepatic binding protein.

The glycoproteins of the present invention include analogs of human erythropoietin with one or more changes in the amino acid sequence of human erythropoietin which result in an increase in the number of sites for sialic acid attachment. These glycoprotein analogs may be generated by site-directed mutagenesis having additions, deletions, or substitutions of amino acid residues that increase or alter sites that are available for glycosylation. Glycoprotein ana-

logs having levels of sialic acid greater than those found in human erythropoietin are generated by adding glycosylation sites which do not perturb the secondary or tertiary conformation required for biological activity. The glycoproteins of the present invention also include analogs having increased levels of carbohydrate attachment at a glycoslyation site which usually involve the substitution of one or more amino acids in close proximity to an N-linked or O-linked site. The glycoproteins of the present invention also include analogs having one or more amino acids extending from the carboxy terminal end of erythropoietin and providing at least one additional carbohydrate site. The glycoproteins of the present invention also include analogs having an amino acid sequence which includes a rearrangement of at least one site for glycosylation. Such a rearrangement of glycosylation site involves the deletion of one or more glycosylation sites in human erythropoietin and the addition of one or more non-naturally occurring glycosylation sites. Increasing the number of carbohydrate chains on erythropoietin, and therefore the number of sialic acids per erythropoietin molecules may confer advantageous properties such as increased solubility, greater resistance to proteolysis, reduced immunogenecity, increased serum half-life, and increased biological activity. Erythropoietin analogs with additional glycosylation sites are disclosed in more detail in European Patent Application 640 619, to Elliot published March 1, 1995.

In a preferred embodiment, the glycoproteins of the present invention comprise an amino acid sequence which includes at least one additional site for glycosylation such as, but not limited to, erythropoietins comprising the sequence of human erythropoietin modified by a modification selected from the following:

$Asn^{30}Thr^{32}$;
$Asn^{51}Thr^{53}$,
$Asn^{57}Thr^{59}$;
Asn^{69};
$Asn^{69}Thr^{71}$;
$Ser^{68}Asn^{69}Thr^{71}$;
$Val^{87}Ans^{88}Thr^{90}$;
$Ser^{87}Asn^{88}Thr^{90}$;
$Ser^{87}Asn^{88}Gly^{89}Thr^{90}$;
$Ser^{87}Asn^{88}Thr^{90}Thr^{92}$;
$Ser^{87}Asn^{88}Thr^{90}Ala^{162}$;
$Asn^{69}Thr^{71}Ser^{87}Asn^{88}Thr^{90}$;
$Asn^{30}Thr^{32}Val^{87}Asn^{88}Thr^{90}$;
$Asn^{89}Ile^{90}Thr^{91}$;
$Ser^{87}Asn^{89}Ile^{90}Thr^{91}$;
$Asn^{136}Thr^{138}$;
$Asn^{138}Thr^{140}$;
Thr^{125}; and
$Pro^{124}Thr^{125}$.

The notation used herein for modification of amino acid sequence means that the position(s) of the corresponding unmodified protein (e.g. hEPO of SEQ ID NO:1 or SEQ ID NO:2) indicated by the superscripted number(s) is changed to the amino acid(s) that immediately precede the respective superscripted number(s).

The glycoprotein may also be an analog having at least one additional amino acid at the carboxy terminal end of the glycoprotein, wherein the additional amino acid includes at least one glycosylation site. The additional amino acid may comprise a peptide fragment derived from the carboxy terminal end of human chorionic gonadotropin. Preferably, the glycoprotein is an analog selected from the group consisting of (a) human erythropoietin having the amino acid sequence, Ser Ser Ser Ser Lys Ala Pro Pro Pro Ser Leu Pro Ser Pro Ser Arg Leu Pro Gly Pro Ser Asp Thr Pro Ile Leu Pro Gln (SEQ ID NO:3), extending from the carboxy terminus; (b) the analog in (a) further comprising Ser^{87} Asn^{88} Thr^{90} EPO; and (c) the analog in (a) further comprising $Asn^{30}Thr^{32}$ Val^{87} Asn^{88} Thr^{90} EPO.

The glycoprotein may also be an analog having an amino acid sequence which includes a rearrangement of at least one site for glycosylation. The rearrangement may comprise a deletion of any of the N-linked carbohydrate sites in human erythropoietin and an addition of an N-linked carbohydrate site at position 88 of the amino acid sequence of human erythropoietin. Preferably, the glycoprotein is an analog selected from the group consisting of Gln^{24} Ser^{87} Asn^{88} Thr^{90} EPO; Gln^{38} Ser^{87} Asn^{88} Thr^{90} EPO; and Gln^{83} Ser^{87} Asn^{88} Thr^{90} EPO.

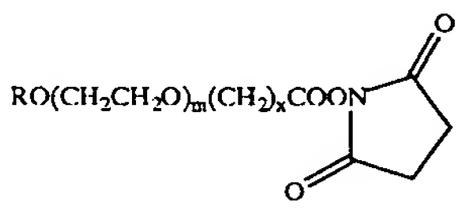
As used herein, "lower alkyl" means a linear or branched alkyl group having from one to six carbon atoms. Examples of lower alkyl groups include methyl, ethyl and isopropyl. In accordance with this invention, R is any lower alkyl. Conjugates in which R is methyl are preferred.

The symbol "m" represents the number of ethylene oxide residues (OCH_2CH_2) in the poly(ethylene oxide) group. A single PEG subunit of ethylene oxide has a molecular weight of about 44 daltons. Thus, the molecular weight of the conjugate (excluding the molecular weight of the EPO) depends on the number "m". In the conjugates of this invention "m" is from about 450 to about 900 (corresponding to a molecular weight of about 20 kDa to about 40 kDa), preferably from about 650 to about 750 (corresponding to a molecular weight of about 30 kDa). The number m is selected such that the resulting conjugate of this invention has a physiological activity comparable to unmodified EPO, which activity may represent the same as, more than, or a fraction of the corresponding activity of unmodified EPO. A molecular weight of "about" a certain number means that it is within a reasonable range of that number as determined by conventional analytical techniques. The number "m" is selected so that the molecular weight of each poly(ethylene glycol) group covalently linked to the erythropoietin glycoprotein is from about 20 kDa to about 4 kDa, and is preferably about 30 kDa.

In the conjugates of this invention, the number "n is the number of polyethylene glycol groups covalently bound to free amino groups (including ϵ-amino groups of a lysine amino acid and/or the amino-terminal amino group) of an erythropoietin protein via amide linkage(s). A conjugate of this invention may have one, two, or three PEG groups per molecule of EPO. "n" is an integer ranging from 1 to 3, preferably "n" is 1 or 2, and more preferably "n" is 1.

The compound of Formula I can be prepared from the known polymeric material:

(II)



in which R and m are as described above, by condensing the compound of Formula II with the erythropoietin glycoprotein. Compounds of Formula II in which x is 3 are alpha-lower alkoxy, butyric acid succinimidyl esters of poly (ethylene glycol) (lower alkoxy-PEG-SBA). Compounds of Formula II in which x is 2 are alpha-lower alkoxy, propionic acid succinimidyl esters of poly(ethylene glycol) (lower alkoxy-PEG-SPA). Any conventional method of reacting an

activated ester with an amine to form an amide can be utilized. In the reaction described above, the exemplified succinimidyl ester is a leaving group causing the amide formation. The use of succinimidyl esters such as the compounds of formula II to produce conjugates with proteins are disclosed in U.S. Pat. No. 5,672,662, issued Sept. 30, 1997 (Harris, et al.).

Human EPO contains nine free amino groups, the amino-terminal amino group plus the ϵ-amino groups of 8 lysine residues. When the pegylation reagent was combined with a SBA compound of Formula II, it has been found that at pH 7.5, a protein:PEG ratio of 1:3, and a reaction temperature of from 20–25° C., a mixture of mono-, di-, and trace amounts of the tri-pegylated species were produced. When the pegylation reagent was a SPA compound of Formula II, at similar conditions except that the protein:PEG ratio was 1:2, primarily the mono-pegylated species is produced. The pegylated EPO can be administered as a mixture, or as the cation exchange chromatography separated different pegylated species. By manipulating the reaction conditions (e.g., ratio of reagents, pH, temperature, protein concentration, time of reaction etc.), the relative amounts of the different pegylated species can be varied.

Human erythropoietin (EPO) is a human glycoprotein which stimulates the formation of erythrocytes. Its preparation and therapeutic application are described in detail for example in U.S. Pat. Nos. 5,547,933 and 5,621,080, EP-B 0 148 605, Huang, S. L., Proc. Natl. Acad. Sci. USA (1984) 2708–2712, EP-B 0 205 564, EP-B 0 209 539 and EP-B 0 411 678 as well as Lai, P. H. et al., J. Biol. Chem. 261 (1986) 3116–3121, an Sasaki, H. et al., J. Biol. Chem. 262 (1987) 12059–12076. Erythropoietin for therapeutic uses may be produced by recombinant means (EP-B 0 148 605, EP-B 0 209 539 and Egrie, J. C., Strickland, T. W., Lane, J. et al. (1986) Immunobiol. 72: 213–224).

Methods for the expression and preparation of erythropoietin in serum free medium are described for example in WO 96/35718, to Burg published Nov. 14, 1996, and in European Patent Publication No. 513 738, to Koch published Jun. 12, 1992. In addition to the publications mentioned above, it is known that a serum-free fermentation of recombinant CHO cells which contain an EPO gene can be carried out. Such methods are described for example in EP-A 0 513 738, EP-A 0 267 678 and in a general form by Kawamoto, T. et al., Analytical Biochem. 130 (1983) 445–453, EP-A 0 248 656, Kowar, J. and Franek, F., Methods in Enzymology 421 (1986) 277–292, Bavister, B., Expcology 271 (1981) 45–51, EP-A 0 481 791, EP-A 0 307 247, EP-A 0 343 635, WO 88/00967.

In EP-A 0 267 678 an ion exchange chromatography on S-Sepharose, a preparative reverse phase HPLC on a C_8 column and a gel filtration chromatography are described for the purification of EPO produced in serum-free culture after dialysis. In this connection the gel filtration chromatography step can be replaced by ion exchange chromatography on S-Sepharose fast flow. It is also proposed that a dye chromatography on a Blue Trisacryl column be carried out before the ion exchange chromatography.

A process for the purification of recombinant EPO is described by Nobuo, I. et al., J. Biochem. 107 (1990) 352–359. In this process EPO is treated however with a solution of Tween® 20, phenylmethylsulfonyl fluoride, ethylmaleimide, pepstatin A, copper sulfate and oxamic acid prior to the purification steps. Publications, including WO 96/35718, to Burg published Nov. 14, 1996, discloses a process for preparing erythropoietin in a serum free fermentation process (EPOsf).

The specific activity of EPO or EPO conjugates in accordance with this invention can be determined by various assays known in the art. The biological activity of the purified EPO proteins of this invention are such that administration of the EPO protein by injection to human patients results in bone marrow cells increasing production of reticulocytes and red blood cells compared to non-injected or control groups of subjects. The biological activity of the EPO proteins, or fragments thereof, obtained and purified in accordance with this invention can be tested by methods according to Annable, et al., Bull. Wld. Hlth. Org. (1972) 47: 99–112 and Pharm. Europa Spec. Issue Erythropoietin BRP Bio 1997(2). Another biological assay for determining the activity of EPO protein, the normocythaemic mouse assay, is described in Example 4. This invention provides a composition comprised of conjugates as described above. A composition containing at least ninety percent mono-PEG conjugates, i.e. in which n is 1, can be prepared as shown in Example 5. Usually mono-PEG conjugates of erythropoietin glycoproteins are desirable because they tend to have higher activity than di-PEG conjugates. The percentage of mono-PEG conjugates as well as the ratio of mono- and di-PEG species can be controlled by pooling broader fractions around the elution peak to decrease the percentage of mono-PEG or narrower fractions to increase the percentage of mono-PEG in the composition. About ninety percent mono-PEG conjugates is a good balance of yield and activity. Sometimes compositions in which, for example, at least ninety-two percent or at least ninety-six percent of the conjugates are mono-PEG species (n equals 1) may be desired. In an embodiment of this invention the percentage of conjugates where n is 1 is from ninety percent to ninety-six percent.

The invention will be better understood by reference to the following examples which illustrate but do not limit the invention described herein.

EXAMPLE 1

Fermentation And Purification Of Human EPO

a) Inoculum Preparation and Fermentation

One vial of the Working Cell Bank, originating from an EPO-producing CHO cell line (ATCC CRL8695, disclosed in EP 411 678 (Genetics Institute) can be used) is taken from the gas phase of the liquid nitrogen storage tank. The cells are transferred into glass spinner flasks and cultivated in a hydrogen carbonate-buffered medium in a humidified CO_2 incubator. Typical serum free media used for the inocolum preparation and fermentation are disclosed in European Patent Application 513 738, to Koch published Jun. 12, 1992, or WO 96/35718, to Burg published Nov. 14, 1996, for example contain as medium DMEM/F12 (e.g. JRH Biosciences/Hazleton Biologics, Denver, US, order No. 57-736) and additionally sodium hydrogencarbonate, L+glutamine, D+glucose, recombinant insulin, sodium selenite, diaminobutane, hydrocortisone, iron(II) sulfate, asparagine, aspartic acid, serine and a stabilizer for mammalian cells such as e.g. polyvinyl alcohol, methyl cellulose, polydextran, polyethylene glycol, Pluronic F68, plasma expander polygelin (HEMACCEL®) or polyvinyl pyrrolidone (WO 96/35718).

The cultures are microscopically checked for the absence of contaminating microorganisms, and the cell densities are determined. These tests are performed at each splitting step.

After the initial growth period, the cell culture is diluted with fresh medium to the starting cell density and undergoes another growth cycle. This procedure is repeated until a culture volume of approximately 2 l per glass spinner flask

has been obtained. After approx. 12 doublings 1 to 5 liter of this culture is available which then is used as inoculum for the 10 l inoculum fermenter.

After 3–5 days, the culture in the 10 l fermenter can be used as inoculum for the 100 l inoculum fermenter.

After additional 3–5 days of cultivation, the culture in the 100 l fermenter can be used as inoculum for the 1000 l production fermenter.

b) Harvesting and Cell Separation

A batch refeed process is used, i.e. when the desired cell density is reached, approx. 80% of the culture is harvested. The remaining culture is replenished with fresh culture medium and cultivated until the next harvest. One production run consists of a maximum of 10 subsequent harvests: 9 partial harvests and 1 overall harvest at the end of fermentation. Harvesting takes place every 3–4 days.

The determined harvest volume is transferred into a cooled vessel. The cells are removed by centrifugation or filtration and discarded. The EPO containing supernatant of the centrifugation step is in-line filtered and collected in a second cooled vessel. Each harvest is processed separately during purification.

A typical process for the purification of EPO-protein is disclosed in WO 96/35718, to Burg published Nov. 14, 1996. The purification process is explained in the following.

a) Blue Sepharose Chromatography

Blue Sepharose (Pharmacia) consists of Sepharose beads to the surface of which the Cibacron blue dye is covalently bound. Since EPO binds more strongly to Blue Sepharose than most non-proteinaceous contaminants, some proteinaceous impurities and PVA, EPO can be enriched in this step. The elution of the Blue Sepharose column is performed by increasing the salt concentration as well as the pH.

The column is filled with 80–100 l of Blue Sepharose, regenerated with NaOH and equilibrated with equilibration buffer (sodium/calcium chloride and sodium acetate). The acidified and filtered fermenter supernatant is loaded. After completion of the loading, the column is washed first with a buffer similar to the equilibration buffer containing a higher sodium chloride concentration and consecutively with a Tris-base buffer. The product is eluted with a Tris-base buffer and collected in a single fraction in accordance with the master elution profile.

b) Butyl Toyopearl Chromatography

The Butyl Toyopearl 650 C (Toso Haas) is a polystyrene based matrix to which aliphatic butyl-residues are covalently coupled. Since EPO binds more strongly to this gel than most of the impurities and PVA, it has to be eluted with a buffer containing isopropanol.

The column is packed with 30–40 l of Butyl Toyopearl 650 C, regenerated with NaOH, washed with a Tris-base buffer and equilibrated with a Tris-base buffer containing isopropanol.

The Blue Sepharose eluate is adjusted to the concentration of isopropanol in the column equilibration buffer and loaded onto the column. Then the column is washed with equilibration buffer with increased isopropanol concentration. The product is eluted with elution buffer (Tris-base buffer with high isopropanol content) and collected in a single fraction in accordance with the master elution profile.

c) Hydroxyapatite Ultrogel Chromatography

The Hydroxyapatite Ultrogel (Biosepra) consists of hydroxyapatite which is incorporated in an agarose matrix to improve the mechanical properties. EPO has a low affinity to hydroxyapatite and can therefore be eluted at lower phosphate concentrations than protein impurities.

The column is filled with 30–40 l of Hydroxyapatite Ultrogel and regenerated with a potassium phosphate/calcium chloride buffer and NaOH followed by a Tris-base buffer. Then it is equilibrated with a Tris-base buffer containing a low amount of isopropanol and sodium chloride.

The EPO containing eluate of the Butyl Toyopearl chromatography is loaded onto the column. Subsequently the column is washed with equilibration buffer and a Tris-base buffer without isopropanol and sodium chloride. The product is eluted with a Tris-base buffer containing a low concentration of potassium phosphate and collected in a single fraction in accordance with the master elution profile.

d) Reversed Phase HPLC on Vydac C4

The RP-HPLC material Vydac C4 (Vydac)consists of silica gel particles, the surfaces of which carry C4-alkyl chains. The separation of EPO from the proteinaceous impurities is based on differences in the strength of hydrophobic interactions. Elution is performed with an acetonitrile gradient in diluted trifluoroacetic acid.

Preparative HPLC is performed using a stainless steel column (filled with 2.8 to 3.2 liter of Vydac C4 silicagel). The Hydroxyapatite Ultrogel eluate is acidified by adding trifluoro-acetic acid and loaded onto the Vydac C4 column. For washing and elution an acetonitrile gradient in diluted trifluoroacetic acid is used. Fractions are collected and immediately neutralized with phosphate buffer. The EPO fractions which are within the IPC limits are pooled.

e) DEAE Sepharose Chromatography

The DEAE Sepharose (Pharmacia) material consists of diethylaminoethyl (DEAE)-groups which are covalently bound to the surface of Sepharose beads. The binding of EPO to the DEAE groups is mediated by ionic interactions. Acetonitrile and trifluoroacetic acid pass through the column without being retained. After these substances have been washed off, trace impurities are removed by washing the column with acetate buffer at a low pH. Then the column is washed with neutral phosphate buffer and EPO is eluted with a buffer with increased ionic strength.

The column is packed with DEAE Sepharose fast flow. The column volume is adjusted to assure an EPO load in the range of 3–10 mg EPO/ml gel. The column is washed with water and equilibration buffer (sodium/potassium phosphate). The pooled fractions of the HPLC eluate are loaded and the column is washed with equilibration buffer. Then the column is washed with washing buffer (sodium acetate buffer) followed by washing with equilibration buffer. Subsequently, EPO is eluted from the column with elution buffer (sodium chloride, sodium/potassium phosphate) and collected in a single fraction in accordance with the master elution profile.

The eluate of the DEAE Sepharose column is adjusted to the specified conductivity. The resulting drug substance is sterile filtered into Teflon bottles and stored at −70° C.

EXAMPLE 2

Pegylation of EPO with mPEG-SBA

EPO purified in accordance with the serum free procedure of Example 1 (EPOsf) was homogeneous as determined by analytical methods and showed the typical isoform pattern

consisting of 8 isoforms. It had a specific biological activity of 190,000 IU/mg as determined by the normocythaemic mouse assay. The pegylation reagent used was a methoxy-PEG-SBA, which is a compound of Formula II in which R is methyl; x is 3; and m is from 650 to 750 (avg. about 680, corresponding to an average molecular weight of about 30 kDa).

Pegylation Reaction

To one hundred milligrams of EPOsf (9.71 ml of a 10.3 mg/ml EPOsf stock, 5.48 μmol) 10 ml of 0.1 M potassium phosphate buffer, pH, 7.5 containing 506 mg of 30 kDa methoxy-PEG-SBA (16.5 μmol) (obtained from Shearwater Polymers, Inc., Huntsville, Ala.) was added and mixed for 2 h at room temperature (20–23° C.). The final protein concentration was 5 mg/ml and the protein:PEG reagent ratio was 1:3. After two hours, the reaction was stopped by adjusting the pH to 4.5 with glacial acetic acid and stored at −20° C., until ready for purification.

Purification

1. Conjugate Mixture: Approximately 28 ml of SP-SEPHAROSE FF (sulfo-propyl cation exchange resin) was packed into an AMICON glass column (2.2x7.5 cm) and equilibrated with 20 mM acetate buffer pH, 4.5 at a flowrate of 150 ml/h. Six milliliters of the reaction mixture containing 30 mg protein was diluted 5-fold with the equilibration buffer and applied onto the column. Unadsorbed materials were washed away with the buffer and the adsorbed PEG conjugate mixture was eluted from the column with 0.175 M NaCl in the equilibration buffer. Unmodified EPOsf still remaining on the column was eluted with 750 mM NaCl. Column was reequilibrated in the starting buffer. Samples were analyzed by SDS-PAGE and their degree of pegylation were determined. It was found that the 0.175M NaCl eluate contained, mono- as well as di- and trace amounts of the tri-pegylated species, whereas the 750 mM NaCl eluate contained unmodified EPOsf.

2. Di-PEG and Mono-PEG-EPOsf: The purified conjugate mixture eluted from the column in the previous step was diluted 4-fold with the buffer and reapplied onto the column and washed as described. Di-PEG-EPOsf and mono-PEG-EPOsf were separately eluted from the column with 0.1M NaCl and 0.175 M NaCl, respectively. Elution was also performed with 750 mM NaCl to elute any remaining unmodified EPOsf.

Alternatively, the reaction mixture was diluted 5-fold with the acetate buffer and applied onto the SP-Sepharose column (~0.5 mg protein/ml gel). Column was washed and adsorbed mono-PEG-EPOsf,di-PEG-EPOsf and unmodified EPOsf were eluted as described in the previous section.

Results

PEG-EPOsf was synthesized by chemically conjugating a linear PEG molecule with a number average molecular weight of 30 kDa. PEG-EPOsf was derived from the reaction between the primary amino groups of EPOsf and the succinimidyl ester derivative of a 30 kDa PEG-butyric acid, resulting in an amide bond.

Results are summarized in Table1. Purified conjugate mixture comprised of mono- and di-PEG-EPOsf and was free of unmodified EPOsf as determined by SDS-PAGE analysis. Conjugate mixture accounted for 23.4 mg or 78% of the starting material. Cation exchange chromatographic separation of mono- and di-PEG-EPOsf indicated that mono- to di-PEG ratio in the conjugate mixture was almost 1:1. After completion of the reaction, ratio of the individual components of Mono: Di: Unmodified were 40:38:20 (%). Overall yield was almost quantitative.

TABLE 1

Summary of results of EPOsf pegylation

Sample	Protein (mg)	Yield (%)
Rxn. Mix.	30	100
Mono-	12.0	40
Di-	11.4	38
Unmod.	6.0	20
Conju. Mix.	23.4	78

EXAMPLE 3

Pegylation of EPO with mPEG-SPA

A different aliquot of the EPOsf used in Example 2 was reacted with 30 kDa methoxy-PEG-SPA (Shearwater Polymers, Inc., Huntsville, Ala.). Reaction was performed at a protein:reagent ratio of 1:2 and purification techniques were in accordance with Example 2. Primarily the mono-pegylated species was produced.

EXAMPLE 4

In-vivo Activity of Pegylated EPO Determined by the Normocythaemic Mouse Assay

The normocythaemic mouse bioassay is known in the art (Pharm. Europa Spec. Issue Erythropoietin BRP Bio 1997 (2)) and a method in the monography of erythropoietin of Ph. Eur. BRP. The samples were diluted with BSA-PBS. Normal healthy mice, 7–15 weeks old, were administered s.c. 0.2 ml of the EPO-fraction containing un-pegylated EPO or tri-, di- or mono-pegylated EPO from Example 2 or 3. Over a period of 6 days, blood was drawn by puncture of the tail vein and diluted such that 1 μl of blood was present in 1 ml of an 0.15 μmol acridine orange staining solution. The staining time was 3 to 10 minutes. The reticulocyte counts were carried out microfluorometrically in a flow cytometer by analysis of the red fluorescence histogram. The reticulocyte counts were given in terms of absolute figures (per 30,000 blood cells analyzed). For the data presented, each group consisted of 5 mice per day, and the mice were bled only once.

In separate experiments, a single dose of unmodified EPO (25 ng of EPO), the PEG(SBA)-EPO mixture from Example 2 (10 ng of conjugate), mono- and di- pegylated EPOs from Example 2 (10 ng of conjugate), the PEG(SPA)-EPO from Example 3 (10 ng of conjugate), and buffer solution were administered to mice. The results are shown in Table 2. The results show the superior activity and the prolonged half life of the pegylated EPO species indicated by the significantly increased amounts of reticulocytes and the shift of the reticulocytes count maximum using the same dose per mouse (10 ng), compared to a dose of 25 ng for unmodified EPO.

TABLE 2

	EPO (Unmodified)	30 kDa SPA PEG	Mono 30 K SBA	Di 30 K SBA	PEG-EPO SBA Conjugate Mixture	Control Buffer
72 h	1000	1393	1411	994	1328	857
96 h	500	1406	1501	926	1338	697
120 h	~200	1100	1182	791	944	701
144 h	~0	535	607	665	660	708

EXAMPLE 5

Preparation of Predominantly mono-PEG-EPO

Pegylation Reaction

Starting with 100 mg (5.48 μmol) of EPOsf in 100 mM potassium phosphate buffer pH 7.5 prepared in accordance with Example 1, there was added 329 mg (10.96 μmol) of 30 kDa PEG-SBA reagent dissolved in 3 ml 1 mM HCl. Enough 100 mM potassium phosphate buffer pH 7.5 was added to make the reaction mixture volume to 20 ml. The final protein concentration was 5 mg/ml and the protein: PEG reagent ratio was 1:2. The reaction mixture was mixed for 2 h at ambient temperature (20–22° C.). After 2 h, the reaction was stopped by adjusting the pH to 4.5 with glacial acetic acid and stored frozen at −20° C. until ready for purification.

Purification

The reaction mixture from the previous step was diluted 1:5 with 10 mM sodium acetate, pH 4.5 and applied to 300 ml SP-Sepharose FF (sulfopropyl cation exchange resin) packed into a 4.2×19 cm column. The column was previously equilibrated with the same buffer. Column effluents were monitored at 280 nm with a Gilson UV monitor and recorded with a Kipp and Zonen recorder. The column was washed with 300 ml or 1 bed volume of equilibration buffer to remove excess reagents, reaction byproducts and oligomeric PEG-EPO. It was followed by washing with 2 bed volumes of 100 mM NaCl to remove di-PEG-EPO. Mono-PEG-EPO was then eluted with 200 mM NaCl. During elution of the mono-PEG-EPO, the first 50 ml of the protein peak was discarded and the mono-PEG-EPO was collected as a 150 ml fraction. Unmodified EPOsf remaining on the column was eluted with 750 mM NaCl. All elution buffers were made in the equilibration buffer. All eluted samples were analyzed by SDS-PAGE and by high performance Size Exclusion Chromatography (SEC). The mono-PEG-EPO pool obtained from the 150 ml fraction, which had no detectable unmodified EPOsf, was then concentrated to ~4.5–7.5 mg/ml and diafiltered into the storage buffer, 10 mM potassium phosphate, 100 mM NaCl , pH 7.5. Concentration/Diafiltration was performed with Millipore Labscale™ TFF System fitted with 50 kDa cut off Millipore Pellicon XL Biomax 50 membrane at ambient temperature. Concentrated mono-PEG-EPO was sterile filtered and stored frozen at −20° C.

Approximately 75% of EPOsf was pegylated. After purification, total yield was ~30% mono-PEG-EPO with no detectable unmodified EPOsf and around 25% di-PEG-EPO. Oligomers, and unpegylated EPOsf accounted for the remaining protein. The mono-PEG-EPO pool obtained from the 150 ml fraction contained approximately 90% mono-PEG-EPO and approximately 10% di-PEG-EPO.

EXAMPLE 6

Thermostability of EPO and Pegylated EPO in Various Formulations: Analysis by DSC (Differential Scanning Calorimetry)

It is generally accepted that the transition temperature of thermal denaturation measured by differential scanning calorimetry is a valid indicator for the thermostability of proteins. Erythropoietin or pegylated erythropoietin (prepared according to Example 3) solutions with concentrations between 0.6 and 1.2 mg/ml were analyzed in various buffers with or without stabilizers by means of a Nano-DSC (Calorimetric Sciences Corporation, Utah, USA) at a heating rate of 2 K/min. An increase in transition temperature indicates an increase in thermal stability of the protein. The measured temperature values should not be understood as absolute values but rather represent differences in the stability of the individual formulations relative to one another.

In order to define the optimal pH of the formulation, the pH-dependence of the thermal denaturation of pegylated erythropoietin in the range between 4 and 9 was studied. The protein samples were analyzed in 30 mM Na_2HPO_4, 30 mM sodium citrate, 30 mM borate. FIG. 1 shows a plateau of maximal transition temperature between about pH 6 to about pH 9 and a sharp decrease below pH 5.5. This indicates that the optimal pH for maximal thermal stability lies above pH 5.5. (FIG. 1).

In order to investigate the effect of ionic strength, the phosphate concentration dependence of thermal denaturation was determined. FIG. 2 shows that the thermal stability increases with an increase in ionic strength of the formulation.

The influence of the buffer substance was also investigated by DSC. From FIG. 3 one can see that the most suitable buffers or additives for a high thermal stability are sulfate, citrate or phosphate. Glycine, which is used as a buffer in currently available formulations (see above) is not very suitable.

FIG. 4 shows that sulfate is also a suitable buffer/additive at low pH (e.g. pH 6.2), whereas phosphate is less suitable at pH 6.2 compared to pH 7.5. This shows that sulfate keeps the thermal stability high, even at low pH. This finding allows a formulation at a pH between 6.0 and 6.5, without severe losses in thermal stability of erythropoietin.

EXAMPLE 7

Aggregation of EPO and peg-EPO Under Thermal Stress: Analysis by SDS-PAGE (Sodium Dodecyl Sulphate Polyacrylamide Gel Electrophoresis)

In order to investigate the effect of heat stress on the erythropoietin protein, samples in different formulations were exposed to heat stress (20 min 80° C.) and analyzed by sodium dodecyl sulphate polyacrylamide gel electrophoresis (SDS-PAGE) under reducing (with DTT in sample buffer) and non-reducing (w/o DTT in sample buffer) conditions. This method allows the detection of covalent aggregate formation. As outlined above, aggregate formation is one of the major degradation pathways of proteins and therefore should be prevented in pharmaceutical formulations of proteins. Aggregates that are detectable in the absence of reducing agent (e.g. DTT) and not detectable in the presence

of reducing agent are highly likely to be formed by incorrect disulfide bridging, an oxidation reaction, under heat stress. FIG. 5 shows the pH dependency of aggregation under heat stress. This experiment clearly shows that the formation of aggregates is suppressed at a pH below 6.5. The higher the pH, the higher the amount of aggregation. Most of the aggregates that are formed can be reduced by treatment of the samples with a reducing agent during SDS-PAGE, suggesting that a great portion of the aggregates that are formed under heat stress are disulfide-bridged dimers, oligomers and higher order aggregates. Taken together, his indicates that the formation of aggregates can be prevented to a great extent by keeping the pH of the formulation at or below pH 6.5.

FIG. 5: Dependency of peg-EPO aggregation on pH. Peg-EPO samples prepared accord to Example 3 were subjected to heat stress (as described above) and then analyzed by SDS-PAGE. Proteins were stained with silver. Lane 1: molecular weight standard. Lane 2: pH 5. Lane 3: pH 5, reduced. Lane 4: pH 6. Lane 5: pH 6, reduced. Lane 6: pH 6.5. Lane 7: pH 6.5, reduced. Lane 8: pH 7. Lane 9: pH 7, reduced. Lane 10: peg-EPO, unstressed.

The formation of aggregates can also be prevented by the use of antioxidants. FIG. 6 shows that the use of 1 mg/ml acetylcysteine as an antioxidant prevents the formation of aggregates under heat stress. Therefore, it is useful to use an antioxidant, like e.g. acetylcysteine at a low pH, e.g. pH 6.2, to prevent aggregate formation under heat stress.

FIG. 6: Peg-EPO aggregation can be prevented by pH 6.2 and/or acetylcysteine. Peg-EPO samples prepared according to Example 3 were subjected to heat stress (as described above) and then analyzed by SDS-PAGE. Proteins were stained with silver. Lane 1: peg-EPO, unstressed. Lane 2: pH 7.5, stressed. Lane 3: pH 6.2, stressed. Lane 4: pH 6.2, stressed, reduced. Lane 5: pH 7.5, 1 mg/ml acetylcysteine, stressed. Lane 6: pH 7.5, 1 mg/ml acetylcysteine, stressed, reduced.

EXAMPLE 8

Stability of peg-EPO in Various Formulations at 4, 25, 30 and 40° C.

Pegylated EPO prepared according to Example 3 in various formulations is incubated at several temperatures. At indicated time points, samples are taken and the stability is assessed by reversed phase high performance chromatography (rpHPLC), high performance size exclusion chromatography (SEC) and sodium dodecyl sulphate polyacrylamide gel electrophoresis (SDS-PAGE). Table 3 compares the stability of peg-EPO in various formulations at several temperatures. These data clearly show the superiority of the herein enclosed formulations regarding protein recovery and aggregation.

TABLE 3

Stability of peg-EPO in various formulations at several temperatures:

For-mulation*	PegEPO (μg/ml)	% recovery after one month at 4° C.	% recovery after one month at 25° C.	% recovery after one month at 30° C.	% recovery after one month at 40° C.	Aggregation at 40° C. detectable (+/-)
A	10	95	92	n.d.	66	-
B	10	93	90	n.d.	64	-
C	10	115	115	111	105	-
D	10	100	99	102	93	-
E	50	n.d.	106	99	84	+
F	50	98	100	98	89	-
G	50	101	101	101	100	-
H	50	105	103	101	102	-
I	50	103	101	104	104	-
A	100	100	99	n.d.	79	+
B	100	103	100	n.d.	77	+
C	100	103	102	103	88	-
D	100	105	106	106	98	-
E	400	98	96	89	88	+
F	400	99	97	96	93	-
G	400	98	96	100	106	-
H	400	107	108	102	97	-
I	400	104	105	98	103	-

*the formulations are:

formulation A: 10 mM sodium phosphate, 100 mM sodium chloride, pH 7.5.

formulation B: 200 mM glycine, pH 7.1.

formulation C: 10 mM sodium phosphate, 140 mM sodium sulfate, pH 6.2.

formulation D: 10 mM sodium phosphate, 40 mM sodium sulfate, 4% (w/v) mannitol, pH 6.2.

formulation E: 10 mM sodium phosphate, 100 mM NaCl, pH 7.0.

formulation F: 10 mM sodium phosphate, 120 mM sodium sulfate, pH 6.2

formulation G: 10 mM sodium phosphate, 40 mM sodium sulfate, 3% (w/v) mannitol, pH 6.2.

formulation H: 10 mM sodium phosphate, 40 mM sodium sulfate, 3% (w/v) mannitol, 7.5 μM $CaCl_2$, pH 6.2.

formulation I: 50 mM arginine, 100 mM sodium sulfate, 1 mM $CaCl_2$, pH 6.2.

SEQUENCE LISTING

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Cys Ser Leu Asn Glu Asn Ile Thr Val Pro Asp Thr Lys Val Asn Phe
35 40 45

Tyr Ala Trp Lys Arg Met Glu Val Gly Gln Gln Ala Val Glu Val Trp
50 55 60

Gln Gly Leu Ala Leu Leu Ser Glu Ala Val Leu Arg Gly Gln Ala Leu
65 70 75 80

Leu Val Asn Ser Ser Gln Pro Trp Glu Pro Leu Gln Leu His Val Asp
85 90 95

Lys Ala Val Ser Gly Leu Arg Ser Leu Thr Thr Leu Leu Arg Ala Leu
100 105 110

Gly Ala Gln Lys Glu Ala Ile Ser Pro Pro Asp Ala Ala Ser Ala Ala
115 120 125

Pro Leu Arg Thr Ile Thr Ala Asp Thr Phe Arg Lys Leu Phe Arg Val
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Tyr Ser Asn Phe Leu Arg Gly Lys Leu Lys Leu Tyr Thr Gly Glu Ala
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Cys Arg Thr Gly Asp
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<210> SEQ ID NO 2
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20 25 30

Cys Ser Leu Asn Glu Asn Ile Thr Val Pro Asp Thr Lys Val Asn Phe
35 40 45

Tyr Ala Trp Lys Arg Met Glu Val Gly Gln Gln Ala Val Glu Val Trp
50 55 60

Gln Gly Leu Ala Leu Leu Ser Glu Ala Val Leu Arg Gly Gln Ala Leu
65 70 75 80

Leu Val Asn Ser Ser Gln Pro Trp Glu Pro Leu Gln Leu His Val Asp
85 90 95

Lys Ala Val Ser Gly Leu Arg Ser Leu Thr Thr Leu Leu Arg Ala Leu
100 105 110

Gly Ala Gln Lys Glu Ala Ile Ser Pro Pro Asp Ala Ala Ser Ala Ala
115 120 125

Pro Leu Arg Thr Ile Thr Ala Asp Thr Phe Arg Lys Leu Phe Arg Val
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Cys Arg Thr Gly Asp Arg
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<210> SEQ ID NO 3
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-continued

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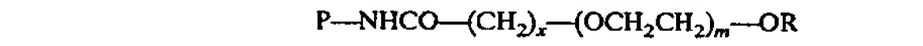
Leu Pro Gly Pro Ser Asp Thr Pro Ile Leu Pro Gln
20 25

What is claimed is:

1. A conjugate comprising an erythropoietin glycoprotein having a free amino group and having the in vivo biological activity of causing bone marrow cells to increase production of reticulocytes and red blood cells and selected from the group consisting of human erythropoietin and analogs thereof which analogs have sequence of human erythropoietin modified by the addition of from 1 to 6 glycosylation sites or a rearrangement of at least one glycosylation site; said glycoprotein being covalently linked to a poly(ethylene glycol) group of the formula —CO—$(CH_2)_x$—$(OCH_2CH_2)_m$—OR by the —CO of said poly(ethylene glycol) group forming an amide bond with said free amino group; wherein R is lower alkyl; x is 2 or 3; m is from about 450 to about 900; and m is chosen so that the molecular weight of the conjugates minus the erythropoietin glycoprotein is from 20 kilodaltons to 100 kilodaltons.

2. The conjugate of claim 1 of the formula:



P—NHCO—$(CH_2)_x$—$(OCH_2CH_2)_m$—OR (I)

wherein m, x and R are as above
and P is the residue of the glycoprotein without the free amino group which forms the amide linkage.

3. The conjugate of claim 2 wherein the glycoprotein is human erythropoietin.

4. The conjugate of claim 3, wherein the human erythropoietin glycoprotein is expressed by endogenous gene activation.

5. The conjugate of claim 3, wherein the glycoprotein has the sequence SEQ ID NO:1.

6. The conjugate of claim 5, wherein R is methyl.

7. The conjugate of claim 5 wherein x is 3.

8. The conjugate of claim 7 wherein said molecular weight is from about 20 kDa to about 4kDa.

9. The conjugate of claim 8 wherein said molecular weight is about 30 kDa.

10. The conjugate of claim 2, wherein the glycoprotein has the sequence of human erythropoietin modified by the addition of from 1 to 6 glycosylation sites.

11. The conjugate of claim 10, wherein the glycoprotein has the sequence of human erythropoietin modified by a modification selected from the group consisting of:
$Asn^{30}Thr^{32}$;
$Asn^{51}Thr^{53}$;
$Asn^{57}Thr^{59}$;
Asn^{69};
$Asn^{69}Thr^{71}$;
$Ser^{68}Asn^{69}Thr^{71}$;
$Val^{87}Asn^{88}Thr^{90}$;
$Ser^{87}Asn^{88}Thr^{90}$;
$Ser^{87}Asn^{88}Gly^{89}Thr^{90}$;
$Ser^{87}Asn^{88}Thr^{90}Thr^{92}$;
$Ser^{87}Asn^{88}Thr^{90}Ala^{162}$;
$Asn^{69}Thr^{71}Ser^{87}Asn^{88}Thr^{90}$;
$Asn^{30}Thr^{32}Val^{87}Asn^{88}Thr^{90}$;
$Asn^{89}Ile^{90}Thr^{91}$;
$Ser^{87}Asn^{89}Ile^{90}Thr^{91}$;
$Asn^{136}Thr^{138}$;
$Asn^{138}Thr^{140}$;
Thr^{125}; and
$Pro^{124}Thr^{125}$.

12. The conjugate of claim 2, wherein the glycoprotein has the sequence of human erythropoietin modified by the rearrangement of at least one glycosylation site.

13. The conjugate of claim 12, wherein the rearrangement comprises deletion of any of the N-linked glycosylation sites in human erythropoietin and addition of an N-linked glycosylation site at position 88 of the sequence of human erythropoietin.

14. The conjugate of claim 13, wherein the glycoprotein has the sequence of human erythropoietin modified by a modification selected from the group consisting of:
$Gln^{24}Ser^{87}Asn^{88}Thr^{90}$;
$Gln^{38}Ser^{87}Asn^{88}Thr^{90}$; and
$Gln^{83}Ser^{87}Asn^{88}Thr^{90}$.

15. The conjugate of claim 14, wherein R is methyl.

* * * * *

UNITED STATES PATENT AND TRADEMARK OFFICE

CERTIFICATE OF CORRECTION

PATENT NO. : 6,583,272 B1
APPLICATION NO. : 09/604938
DATED : June 24, 2003
INVENTOR(S) : Pascal Sebastian Bailon

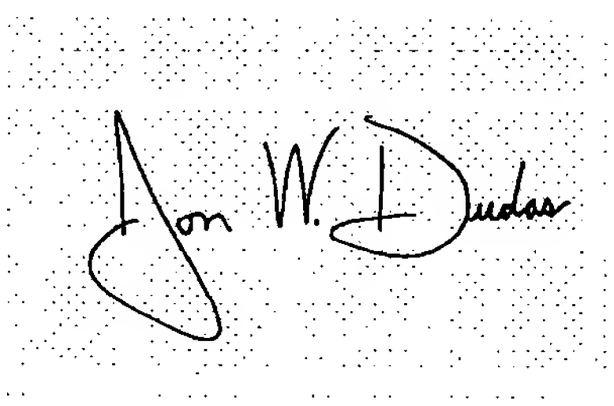
It is certified that error appears in the above-identified patent and that said Letters Patent is hereby corrected as shown below:

IN THE CLAIMS:

Column 19, line 48: "4kDa" should read -- 40kDa -- .

Signed and Sealed this

Twenty-second Day of May, 2007



JON W. DUDAS
Director of the United States Patent and Trademark Office

D

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SOUTHFIELD MI 48034-1095

MAINTENANCE FEE STATEMENT

According to the records of the U.S.Patent and Trademark Office (USPTO), the maintenance fee and any necessary surcharge have been timely paid for the patent listed below. The "PYMT DATE" column indicates the payment date (i.e., the date the payment was filed).

The payment shown below is subject to actual collection. If the payment is refused or charged back by a financial institution, the payment will be void and the maintenance fee and any necessary surcharge unpaid.

Direct any questions about this statement to: Mail Stop M Correspondence, Director of the USPTO, P.O.Box 1450, Alexandria, VA 22313-1450.

PATENT NUMBER	FEE AMT	SUR CHARGE	PYMT DATE	U.S. APPLICATION NUMBER	PATENT ISSUE DATE	APPL. FILING DATE	PAYMENT YEAR	SMALL ENTITY?	ATTY DKT NUMBER
6,583,272	**$900.00**	**$0.00**	**11/16/06**	**09/604,938**	**06/24/03**	**06/27/00**	**04**	**NO**	**1097**

EXHIBIT E-1

December 4, 2001

Food and Drug Administration
Center for Biologic Evaluation and Research
Office of Therapeutics Research and Review
c/o Document Control Room, HFM-99
12100 Parklawn Drive, 1st Floor
Rockville, MD 20852

Ladies and Gentlemen:

Re: Initial Investigational New Drug Application (S-000)
Ro 50-3821: Pegylated Epoetin beta (methoxy polyethylene glycol-epoetin beta)
Intravenous and Subcutaneous Administration
For the Treatment of Anemia Associated with Chronic Renal Failure

Pursuant to Section 312.20 of Title 21 of the Code of Federal Regulations, Hoffmann-La Roche Inc., hereby submits, in triplicate, the Investigational New Drug Application (IND) for Ro 50-3821. This agent is administered both intravenously and subcutaneously, and will be investigated for treatment of patients with anemia associated with chronic renal failure.

Background

Ro 50-3821 is a mono-pegylated form of epoetin beta, a recombinant human erythropoietin. The pharmacological action of Ro 50-3821 is identical to that of erythropoietin beta in binding to surface receptors of erythroid progenitor cells to trigger proliferation, maturation and terminal differentiation of colony-forming units.

The CMC section (IND Item 7) presents a summary of the development, current manufacturing and control procedures and data supporting the quality and stability for the methoxy polyethylene glycol-epoetin beta drug substance and the final formulated drug product, Ro 50-3821. The non-clinical toxicology program of Ro 50-3821 is designed to provide adequate safety information for an IND application to undertake clinical investigations for treatment of anemia in chronic renal failure patients. Tabulated summaries of key findings are presented in this initial IND Item 8.

Information about safety, tolerability, pharmacokinetics and pharmacodynamics of Ro 50-3821 has been collected in five Phase I studies conducted outside the United States. The Phase I program includes the following: two completed single ascending dose studies (Study BP16198 and BP16239), two ongoing multiple ascending dose studies, one with intravenous administration (BP16346) and one with subcutaneous administration (WP16422) and one completed cross-over study (WP16383). An overall summary report and corresponding individual reports of these Phase I studies are included in Item 9 of this IND.

Planned Phase II Program

The planned Phase II program will include 4 studies, two to establish starting doses for correction of renal anemia in epoetin native patients (BA16260 and BA16528) and two to determine conversion factors for switching treatment from exogenous epoetin to Ro 50-3821 (BA16285 and BA16286). Study BA16285 will be conducted in the US only; studies BA16528 and BA16286 will include both US and European centers. These three studies will be conducted under US IND. Full protocols for these studies are included within Item 6 of this IND. In accord with local country laws, three EU centers of study BA16286 have just been initiated and enrollment has commenced. Study BA16260 will only be conducted in Europe and the protocol is provided in the IND for informational purposes only; six centers have been initiated with 17 patients randomized to date.

Hoffmann-La Roche Inc. 340 Kingsland Street
Nutley, New Jersey 07110-1199

Previous Discussions with CBER

A preIND teleconference was held with members of CBER and Hoffmann-La Roche Inc. on November 1, 2001 to discuss our proposed clinical development program for Ro 50-3821 as outlined in a September 28, 2001 preIND Briefing Package. The below summarizes those discussions and agreements relative to this initial IND filing:

Chemistry, Manufacturing and Controls

1. FDA requested and Roche agreed that the following CMC items would be addressed in the IND:
   - Lot release and specifications for the drug product and drug substance
   - Quantification of viral load, viral clearance calculation and resulting virus load per dose of drug product
   - Safety evaluation for raw materials of biological origin. (Note that there are no raw materials of human or animal origin used in the production of Ro 50-3821)
   - Stability data (data to be presented include one 400 mcg/mL lot at 12 months, one 400 mcg/mL lot at 6 months and one 100 mcg/mL lot at 6 months stability)
   - A discussion of the sites of pegylation on the epoetin beta molecule

Preclinical

2. FDA acknowledged that judging by the information furnished in the IB, preclinical safety evaluation is extensive and satisfactory to support clinical trials. Roche confirmed that all final study reports for the preclinical program would be submitted in the IND. It was also confirmed that the initial IND would contain 3 month toxicology data for Ro 50-3821.
3. For labeling purposes, FDA suggested that Roche should consider repeating reproductive toxicity studies with Ro 50-3821. Roche indicated that the justification to use epoetin beta data is provided in the Toxicology Summary of this IND (as previously agreed with the reviewer in teleconferences on September 20, 2000 and September 27, 2000; see last paragraph of this letter). FDA agreed to have further discussions on this topic after their review of Roche's justification.
4. FDA requested and Roche confirmed that epoetin beta mutagenicity studies will be included in the initial IND.
5. FDA requested anti-EPO antibody assay for preclinical and clinical samples to be included in the IND. Roche indicated that for preclinical samples, the method description is included in each toxicology reports. In addition, a general description for clinical samples will be provided in the initial IND.
6. FDA requested Ro 50-3821 binding profile data in human tissues for the BLA filing. Roche indicated that relevant data exist for epoetin beta and will be included in the initial IND. FDA acknowledged but further suggested that Roche should provide comparable data with Ro 50-3821 in the BLA filing, as a basis for supporting the rationale for not performing carcinogenicity studies. FDA requested and Roche confirmed that the mouse carcinogenicity study with recombinant mouse EPO will be included in the initial IND.

Clinical Phase II Protocols:

7. The FDA requested and Roche agreed to specify stopping rules regarding safety for the Phase II protocols.
8. FDA suggested and Roche agreed that the duration of the phase II studies should be extended from 15 weeks to a longer period (due to red blood cells' long life span). This suggestion was made to give more complete information on individual patients with a dose change within the study period.
9. Regarding the DSMB, the FDA requested and Roche agreed to provide an explanation within the initial IND for the DSMB's function, its composition (including CVs), confirmation of its independence from the sponsor, a description of the communication process between the DSMB and the Sponsor, and the frequency of its meetings. The FDA also requested that safety monitoring by the DSMB should be done on an ongoing basis.

10. For the Phase II protocols, FDA recommended and Roche agreed that the ITT population should be defined as all patients randomized, not all patients randomized who received at least one dose of study drug.
11. The FDA requested and Roche agreed to further consider the individual dose adjustments in the Phase II protocols, where 50% dose increases were recommended in the protocols supplied in the preIND package. FDA indicated that smaller increments may be more appropriate and any proposed adjustments should be justified or decreased depending on Phase I data, specifically MAD, and presented in the initial IND.
12. FDA agreed to the proposed scheme for dose escalation between treatment groups.
13. FDA agreed to the ECG monitoring assessments proposed for Phase II. FDA also agreed that the ECG information from Phase I as presented in the pre-IND package, including the two summary reports by Professor Camm, an external cardiology expert, was acceptable for the IND filing. FDA acknowledged that they received the Sponsor's facsimile of October 31, 2001 regarding the patient with a prolonged PR interval; they noted they did not have a chance to review the case before the teleconference. No immediate concerns were communicated.
14. FDA requested and Roche agreed to include in the initial IND pharmacokinetic(PK), pharmacodynamic (PD) and full safety data from the 1.6 iv dose group from the ongoing MAD study BP16346. Other data from Phase I studies to be included in the initial IND are: SAD studies (BP16198 and BP16329) PK/PD and safety for all dose groups, cross-over study WP16383 to include PK/PD and safety for all dose groups, MAD (sc, WP16422) 0.4 and 0.8 dose groups with PK/PD and final safety, and final safety for the 1.6 group. FDA agreed to the Sponsor's proposal that the ongoing MAD studies can be presented in the IND as abbreviated reports. Furthermore, the FDA stated that the final study results of the ongoing Phase I studies can be submitted when the studies are completed.

Three additional teleconferences were held between Roche and Dr. Mercedes Serabian regarding the preclinical program on September 20, 2000, September 26, 2000 and September 27, 2000. The following summarizes the discussions and agreements reached during these teleconferences:

- Roche will provide full information up to 3 months duration on toxicity, toxicokinetics, weekly monitoring of PD markers, antibody assay and its reversibility in two species by both intravenous and subcutaneous administration
- Roche will evaluate chronic toxicity in rats by the subcutaneous administration for six month duration. Phase II clinical studies of 4 months duration can be started with 3 months toxicity data, and ongoing 6 month study data will be provided to the IND after completion of the study.
- Information from the 13-week toxicity studies demonstrates all animals receiving Ro 50-3821 will eventually become either severely anemic or polycythemic depending on individual susceptibility to antibody development. Considering the poor clinical conditions, including mortality, that both anemia and polycythemia imposed on test animals, it is not considered ethical to conduct the study with longer duration than 13-weeks in dogs.
- Roche will submit mutagenicity, carcinogenicity and reproductive toxicity reports from epoetin beta with the submission of the initial IND.

This submission contains 80 volumes. Please refer to the table of contents for the location of detailed information.

If you have any questions regarding this application or require any additional information, please contact the undersigned at the numbers provided.

We understand that this Investigational New Drug Application and all information contained therein, unless otherwise made public by Hoffmann-La Roche Inc., is confidential. Additionally, certain pages herein are marked "CONFIDENTIAL" because the information therein constitutes trade secrets or information which is privileged or confidential within the meaning of the Freedom of Information Act (5 USC 552) and will remain so subsequent to approval of the NDA for this drug. If for any reason Food and Drug Administration officials should at any time feel that disclosure of any of the materials marked "CONFIDENTIAL" should be made to any member of the public, we expect that because of the importance of maintaining confidentiality of these materials to Hoffmann-La Roche Inc., you will first consult us on the issue of disclosure.

Sincerely,

HOFFMANN-LA ROCHE INC.

Lisa Ann Luther
Group Director
Drug Regulatory Affairs
(973) 562-3679 - Phone
(973) 562-3700 - Fax

LAL/js

HLR No. 2001-2752
Attachments

EXHIBIT E-2

DEPARTMENT OF HEALTH & HUMAN SERVICES

Food and Drug Administration
1401 Rockville Pike
Rockville MD 20852-1448

Our Reference: BB-IND 10158

DEC 17 2001

Hoffmann-La Roche, Incorporated
Attention: Ms. Lisa Ann Luther
Group Director Drug Regulatory Affairs
340 Kingsland Street
Nutley, NJ 07110

Dear Ms. Luther:

The Center for Biologics Evaluation and Research has received your **Investigational New Drug Application (IND)**. The following product name and BB-IND number have been assigned to this application. They serve only to identify it and do not imply that this Center either endorses or does not endorse your application.

BB-IND #: 10158

SPONSOR: Hoffmann-La Roche, Incorporated

PRODUCT NAME: Pegylated Erythropoietin beta (human, recombinant, CHO cells, Hoffmann-La Roche)

DATE OF SUBMISSION: December 4, 2001

DATE OF RECEIPT: December 7, 2001

This BB-IND number should be used to identify all future correspondence and submissions, as well as telephone inquiries concerning this IND. Please provide an **original and two copies of every submission to this file**. Please include three originals of all illustrations which do not reproduce well.

It is understood that studies in humans will not be initiated until 30 days after the date of receipt shown above. If this office notifies you, verbally or in writing, of serious deficiencies that require correction before human studies can begin, it is understood that you will continue to withhold such studies until you are notified that the material you have submitted to correct the deficiencies is satisfactory. If such a clinical hold is placed on this file, you will be notified in writing of the reasons for placing the IND on hold.

You are responsible for compliance with applicable portions of the Public Health Service Act, the Federal Food, Drug, and Cosmetic Act, and the Code of Federal Regulations (CFR). A copy of 21 CFR Part 312, pertaining to INDs, is enclosed. Copies of other pertinent regulations are available from this Center upon request. The following points regarding obligations of an IND sponsor are included for your information only, and are not intended to be comprehensive.

Progress reports are required at intervals not exceeding one year and are due within 60 days of the anniversary of the date that the IND went into effect [21 CFR 312.33]. Any unexpected, fatal or immediately life-threatening reaction associated with use of this product must be reported to this Division by telephone or facsimile transmission no later than seven calendar days after initial receipt of the information. All serious, unexpected adverse experiences, as well as results from animal studies that suggest significant clinical risk, must be reported, in writing, to this Division and to all investigators within fifteen calendar days after initial receipt of this information [21 CFR 312.32].

Charging for an investigational product in a clinical trial under an IND is not permitted without the prior written approval of the FDA.

Prior to use of each new lot of the investigational biologic in clinical trials, please submit the lot number, the results of all tests performed on the lot, and the specifications when established (i.e., the range of acceptable results).

If not included in your submission, please provide copies of the consent forms for each clinical study. A copy of the requirements for and elements of informed consent are enclosed. Also, please provide documentation of the institutional review board approval(s) for each clinical study.

All laboratory or animal studies intended to support the safety of this product should be conducted in compliance with the regulations for "Good Laboratory Practice for Nonclinical Laboratory Studies" (21 CFR Part 58, copies available upon request). If such studies have not been conducted in compliance with these regulations, please provide a statement describing in detail all differences between the practices used and those required in the regulations.

Item 7a of form FDA 1571 requests that either an "environmental assessment," or a "claim for categorical exclusion" from the requirements for environmental assessment, be included in the IND. If you did not include a response to this item with your application, please submit one. See the enclosed information sheet for additional information on how these requirements may be addressed.

Telephone inquiries concerning this IND should be made directly to me at (301) 827-4358. Correspondence regarding this file should be addressed as follows:

Center for Biologics Evaluation and Research
Attn: Office of Therapeutics Research and Review
HFM-99, Room 200N
1401 Rockville Pike
Rockville, MD 20852-1448

If we have any comments after we have reviewed this submission, we will contact you.

Sincerely yours,

Karen D. Winestock

Karen D. Winestock
Regulatory Project Manager
Division of Application Review and Policy
Office of Therapeutics
Research and Review
Center for Biologics
Evaluation and Research

Enclosures (3): 21 CFR Part 312
21 CFR 50.20, 50.25
Information sheet on 21 CFR 25.24

HLR # 2008-28

EXHIBIT F-1

April 18, 2006

Dr. George Q. Mills
Division of Hematology Products
Office of Oncology Drug Products
Center for Drug Evaluation and Research
Food and Drug Administration
Therapeutic Biological Products Document Room
5901-B Ammendale Road
Beltsville, Maryland 20705-1266

Re: MIRCERA® (pegserepoetin alfa, RO0503821), for the treatment of anemia associated with chronic kidney disease, including patients on dialysis and not on dialysis
Original Biologic License Application (BLA): STN 125164/0

Dear Dr. Mills:

In accordance with 21 CFR Part 314.50, Hoffmann-La Roche Inc. (Roche) herewith submits an original Biologics License Application (BLA) supporting the use of MIRCERA® for the treatment of anemia associated with chronic kidney disease (CKD) including patients on dialysis and not on dialysis. MIRCERA® has been the subject of BB-IND 10158 in renal anemia and BB-IND 10964 for the treatment of anemia in cancer patients receiving chemotherapy.

The proposed starting dose in patients not currently treated with an erythropoiesis stimulating agent (i.e. the correction setting) is 0.6 µg/kg IV or SC administered as a single dose once every two weeks. The proposed starting dose for patients converting from epoetin alfa or darbepoetin alfa (i.e. maintenance setting) is based on the total weekly epoetin or darbepoetin dose at the time of conversion, administered once monthly or, if desired, once every two weeks as an IV or SC injection as described below:

Conversion from Epoetin Alfa

Previous Weekly Epoetin Alfa Dose (Units/week)	MIRCERA® Dose	
	Once Monthly (µg/month)	Once Every Two Weeks (µg/q2w)
<8000	120	60
8000-16000	200	100
>16000	360	180

Conversion from Darbepoetin Alfa

Previous Weekly Darbepoetin Alfa Dose (µg/week)	MIRCERA® Dose	
	Once Monthly (µg/month)	Once Every Two Weeks (µg/q2w)
<40	120	60
40-80	200	100
>80	360	180

Hoffmann-La Roche Inc. 340 Kingsland Street
Nutley, New Jersey 07110-1199

Background and Overview of the Clinical Development Program

The clinical development program for MIRCERA® consists of 13 Phase I clinical pharmacology studies, 4 Phase II clinical studies and 6 pivotal Phase III studies. The 13 clinical pharmacology studies comprised of 499 healthy volunteers, 24 CKD patients not on dialysis and 16 patients on peritoneal dialysis. In addition, 3 population pharmacokinetic and pharmcodynamic analyses were performed based on samples from over 500 CKD patients in the clinical program.

The Phase II program consisted of two correction studies (BA16260 and BA16528) and two maintenance studies (BA16285 and BA16286). All four studies were open label, randomized, multicenter dose finding studies to establish the starting doses of MIRCERA® .

The Phase III program consisted of two correction studies (BA16736 and BA16738) and four maintenance studies (BA16739, BA16740, BA17283, BA17284). The two correction studies were randomized, open-label, multicenter studies, with the primary objective to demonstrate the efficacy of MIRCERA® treatment in the correction of anemia based on hemoglobin response rate. In addition to hemoglobin response rate, study BA16738 had a noninferiority evaluation. The four maintenance studies were open-label, randomized, multicenter, non-inferiority studies with the primary objective of showing that MIRCERA® can maintain hemoglobin concentrations when converting from an erythropoiesis stimulating agent (ESA). Study BA16739 and BA16740 evaluated conversion from epoetin whereas Study BA17283 evaluated conversion from darbepoetin alfa. Study BA17284 was conducted to support registration of prefilled syringes as an alternative presentation to vials.

The six Phase III studies have allowed an assessment of the efficacy and safety of MIRCERA® including different routes of administration (IV and SC), dosing intervals (once every two weeks and once every four weeks), presentations (vials, pre-filled syringe), and across stages of CKD (patients on dialysis and patients not on dialysis). Studies have been conducted in both anemia correction (patients not currently treated with an ESA) and maintenance settings (patients converting from current treatment with either epoetin or darbepoetin alfa).

The safety data in the BLA include data from the four Phase II studies and six Phase III studies involving a total of 2,737 patients who received treatment with MIRCERA® or a reference comparator. In addition to these 10 studies in patients, the Integrated Summary of Safety (ISS) includes supportive safety data from clinical pharmacology studies (Phase I studies), two Phase IIIb extension studies in CKD patients on dialysis and not on dialysis, Phase I/II and Phase II studies in the oncology setting and studies to support a Japanese development program.

CMC Development Program

Pegserepoetin alfa is a polymer-based erythropoietic compound which is formulated as a sterile, preservative-free protein solution for intravenous (IV) or subcutaneous (SC) administration. The drug substance is commonly referred to (or can be described) as methoxy polyethylene glycol-epoetin beta. The drug substance is synthesized by chemically combining one linear methoxy polyethylene glycol molecule (PEG), with an average molecular weight of around 30 kDa, to epoetin beta (EPO, RO2053859). This results in a molecular weight of around 60 kDa.

Hoffmann-La Roche Inc. 340 Kingsland Street
Nutley, New Jersey 07110-1199

The drug substance process has evolved over time in four main steps (process variants) designated as preliminary process, 1F process, 2F process and 3F process. Beginning with the 1F process (20 g scale/pilot facility), the final purification process was implemented. This included a change in the chromatography column matrix with two sequential chromatography steps implemented to improve the purity of the drug substance. The pegylation reaction did not change. The final concentrated drug substance is dialyzed in a sodium phosphate buffer of pH that is consistent with the final drug product formulation. This step was also implemented with the 1F process. The 1F process material was used through Phase II clinical trials. Phase II (extension studies) and Phase III clinical trials used drug substance made by the 2F process (60 g scale/pilot facility). The 3F batches (60 g scale/commercial facility) are the registration batches. The manufacturing process for the EPO starting material did not change throughout the clinical trials.

In parallel to the implementation of the 1F process for drug substance, the drug product formulation was changed. To bridge the preclinical and early clinical trial material to the drug substance final process and final drug product formulation, an analytical comparability assessment was done along with preclinical toxicology studies and a relative bioavailability study.

Pegserepoetin alfa drug product is intended to be marketed in two dosage presentations; vials and pre-filled syringes (PFS). The drug product solutions of each dosage strength are identical in their qualitative composition. Quantitatively, they differ in their content of drug substance according to the dosage strengths.

Seven dosage strengths for the pegserepoetin alfa vials are intended for registration:
50 µg/ mL, 100 µg/ mL, 200 µg/ mL, 300 µg/ mL, 400 µg/ mL, 600 µg/ mL and 1000 µg/ mL

Nine dosage strengths for the pegserepoetin alfa prefilled syringes are intended for registration:
50 µg/ 0.3 mL, 75 µg/ 0.3 mL, 100 µg/ 0.3 mL, 150 µg/ 0.3 mL, 200 µg/ 0.3 mL, 250 µg/ 0.3 mL, 400 µg/ 0.6 mL, 600 µg/ 0.6 mL, 800 µg/ 0.6 mL.

Nonclinical Development Program

The nonclinical development program was conducted under BB-IND 10158. The preclinical program documented in this BLA comprises 13 pharmacology studies, 5 pharmacokinetic studies, 8 toxicology studies, 6 reproductive studies and 3 local tolerance studies.

The nonclinical safety program for MIRCERA® consisted of two single dose acute toxicity studies in mice and rats, four 13-week toxicity studies in two species (rats and dogs) by either IV or SC routes of administration, and a 26-week chronic toxicity study in rats by SC administration. A cardiovascular safety pharmacology study in dogs by IV administration and a complete battery of reproductive studies in rats and rabbits by SC administration were also conducted. Both intended clinical routes, IV and SC, were tested in 13-week studies to assess if differences in toxicokinetics and/or toxicity profiles might occur via different routes of administration.

As previously agreed with FDA, in lieu of formal carcinogenicity studies, an in vitro cell proliferation study was conducted in order to assess whether MIRCERA® could stimulate proliferation of non-target human cells. In addition, a tissue binding study was conducted to evaluate the ability of MIRCERA® to bind cell surface receptors on non-target human tissues, and subsequently it's potential for growth stimulation of non-target cells.

FDA/Roche Key Meetings/Teleconferences and Agreements

Roche has been in continuous dialogue with FDA on the development program for MIRCERA®. Key interactions with FDA included an End-of-Phase II clinical and CMC meeting as well as a Clinical and CMC Pre-BLA meeting. In addition, Roche has engaged in numerous teleconferences during the development of MIRCERA®.

FDA feedback on the nonclinical and clinical pharmacology programs were discussed at the End-of-Phase II meeting. In addition, feedback from FDA included the content/format of the BLA (December 2005) and the pediatric program to support the Pediatric Research Equity Act of 2003 (February 2006). A clinical pre-BLA meeting was held on March 6, 2006 where FDA noted that the overall safety and efficacy results support a BLA filing.

Attachment 1 of this cover letter provides an overview of the key agreements with FDA during the development program of MIRCERA®.

BLA Content/Format

As agreed with FDA, this original BLA is being submitted using the e-BLA folder structure and item Table of Contents (TOC) with Common Technical Document (CTD) formatted documents. A Reviewer's Guide is provided as attachment 2 of this cover letter in order to facilitate the review of the BLA.

Labeling:

The proposed package insert (PI), patient package insert (PPI) and proposed labeling for the packaging components for vials and prefilled syringes are included in Item 2 of the application. In addition, the proposed PI is also provided in SPL format.

Risk Management Plan:

The objective of the Risk Management Plan (RMP) is to outline the pharmacovigilance activities associated with the use of MIRCERA® in the treatment of anemia associated with chronic kidney disease (CKD). The RMP summarizes the identified risks of MIRCERA® in clinical trials and the plans to manage the identified and potential risks known with ESAs. This RMP appears in Item 8 of the BLA.

Compliance with Pediatric Research Equity Act of 2003:

In accordance with the pediatric research equity act of 2003, Roche submitted to BB-IND 10158, a pediatric development plan, a request for waiver and deferral of pediatric assessment for MIRCERA® (S-198). FDA granted a waiver of pediatric assessment in neonates up to age of 5 as well as a deferral of pediatric assessment for ages 5 – 16. In addition, FDA noted that with acceptable study modifications, the proposed clinical studies represented a reasonable approach to fulfilling the requirements of the Pediatric Research Equity Act of 2003 (February 2006).

Based on the FDA recommendations, Roche intends to revise the pediatric development plan and submit protocols for FDA review to BB-IND 10158 by November 2006.

Proprietary (trade) and Non-Proprietary Names:

The proprietary (trade) name of MIRCERA® was submitted to the Division for review on April 5, 2006 (S-235). The non-proprietary name is pegserepoetin alfa. Final approval of the non-proprietary name is pending.

Environmental Assessment:

Roche claims a categorical exclusion from the requirement to prepare an environmental assessment in accordance with 21 CFR 25.31(c). Additional information is found in the regional information section of Item 4 (CMC) of the BLA.

Overall Table of Contents of BLA:

This BLA is organized as follows:

Item	Documents	Location
Item 1	Form 356h, Cover letter and TOC	DLT Tape 1
Item 2	Labeling	DLT Tape 1
Item 3	Summary	DLT Tape 1
Item 4	Chemistry, Manufacturing and Controls (CMC)	DLT Tape 1
Item 5	Nonclinical Pharmacology and Toxicology	DLT Tape 1
Item 6	Human pharmacology and bioavailabilty/bioequivalence	DLT Tape 1
Item 7	Clinical Microbiology	Not Applicable
Item 8	Clinical	DLT Tape 1
Item 9	Safety Update Report	Not Applicable
Item 10	Statistical	DLT Tape 1
Item 11	Case Report Tabulations	DLT Tape 1
Item 12	Case Report Forms	DLT Tape 1
Item 13	Patent Information	DLT Tape 1
Item 14	Patent Certification	Not Applicable
Item 15	Establishment Description	DLT Tape 1
Item 16	Debarment certification	DLT Tape 1
Item 17	Field Copy certification[a]	DLT Tape 1
Item 18	Use Fee Cover Sheet	DLT Tape 1
Item 19	Financial Information	DLT Tape 1
Item 20	Other	DLT Tape 1

[a] As agreed with FDA on March 30, 2006, a field certification letter is not being submitted to the FDA district office since the DMPQ/TFRB Group at FDA will review the CMC section of the BLA to facilitate the prior approval inspection and not the district office.

This BLA is comprised of 1 DLT table totaling approximately 10 GB. The DLT tape has been scanned with Symantec AntiVirus 9.0.5.1000 virus definition updated 4/13/2006 rev. 7 and no viruses were found. As discussed with Ms. Florence Moore, 2 electronic archive copies (a total of 2 DLT tapes) are being provided. All original signature forms/pages are provided as paper in one volume as Official Archive Copy. These include: the cover letter, Form 356h, User Fee Cover Sheet Form 3397 and Financial Information Forms 3454 and 3455.

Confidential Information:

Since this Biologic License Application has not yet been approved, this submission is considered as constituting trade secrets or commercial or financial information, which is privileged or confidential within the meaning of the Freedom of Information Act (5 USC 552). It is requested that this submission not be published until the Biologics License Application has been approved.

Contact Information:

In order to facilitate the review, we encourage FDA to contact us to clarify any issues or address any questions. Please contact Ms. Susan Batcha at (973) 562-3514 or Ms. Deborah Savuto at (973) 562-3705 with any MIRCERA® chemistry, manufacturing and controls issues; Dr. Krishnan Viswanadhan at (973) 235-6241 or Dr. Jennifer Dudinak at (973) 562-2930 for all other issues.

Please do not hesitate to contact the undersigned for any further information or clarification. Roche looks forward to your review and approval of this application.

Sincerely,

HOFFMANN-LA ROCHE INC.

Krishnan Viswanadhan, Pharm.D.
Associate Director
Drug Regulatory Affairs
(973) 235 – 6241 (telephone)
(973) 562 - 3700 (fax)

Desk Copy: Dwaine Rieves – Cover Letter only
Jong-Hoon (John) Lee – Cover Letter only
Florence Moore – Cover Letter only

KV/jm
Attachments (2)
HLR No. N2006-01194

Attachment 1: Key Agreements with FDA

KEY AGREEMENTS ON PRECLINICAL DEVELOPMENT PROGRAM

The following key agreements were made with FDA on the preclinical development program at the pre-IND meeting on November 27, 2001 and the end of phase II meeting on October 2, 2003:

- FDA noted that the studies were very extensive. However, FDA recommended that reproductive toxicity studies be performed.

In accordance with FDA's recommendation, a package of reproductive testing consisting of fertility, embryofetal development, prenatal and postnatal development studies were conducted.

- FDA agreed that the preclinical ADME program was acceptable. FDA also noted that mass balance studies would not be required for registration

- FDA agreed that the in vitro cell proliferation study and human tissue binding study to evaluate the carcinogenic potential of MIRCERA® in lieu of formal carcinogenicity studies would be acceptable.

- FDA agreed that the preclinical toxicology program looked adequate to support phase II and BLA submission.

KEY AGREEMENTS ON CLINICAL PHARMACOLOGY DEVELOPMENT PROGRAM

- FDA agreed with the overall clinical pharmacology program. FDA noted that final decision on acceptability will depend on data submitted.

- FDA agreed with Roche's evaluation of pharmacokinetic variability

- FDA suggested that population pharmacokinetics also be considered in the phase III predialysis study.

The phase II population pharmacokinetic analysis included data from the phase II predialysis study.

- FDA agreed with the proposal to not conduct formal drug-drug interaction studies but to use a population pharmacokinetic approach to explore the effect of other drugs on the PK and PD of MIRCERA®. However, FDA noted that if the results from the exploratory analysis suggest there is a drug interaction, formal interaction studies might be needed.

Since no drug interactions were seen based on the population pharmacokinetic approach, no formal pharmacokinetic drug interaction study was performed.

KEY AGREEMENTS ON CMC PROGRAM

Key agreements on the chemistry manufacturing and controls were made with FDA at the CMC End of phase II meeting as well as the CMC Pre-BLA meeting. The key agreements were as follows:

CMC End of Phase II Meeting:

- Agreement with FDA that the drug substance made at the 60 g scale is analytically comparable to drug substance made at previous production scales (3g, 7.5g, and 20g) based on data derived from routine release testing, extended characterization testing, and functional assay (bioassay).

- FDA agreed with the approach for licensing the commercial facility for the production of RO0503821 drug substance based on analytical comparability.

- FDA agreed that the proposed bracketing program for registration and stability of RO0503821 drug product is acceptable.

CMC Pre-BLA Meeting:

FDA noted that pending review of the BLA, the following was acceptable:

- Inclusion in the BLA of building 354 as a second production site of EPO starting material

- The proposed approach for the drug product validation matrix plan

- The proposed approach for bulk sterility exemption for drug product

- Inclusion in the BLA of a comparability protocol for post approval changes

- Amending the BLA to include the additional stability data without extending the review clock.

- FDA agreed that it was unnecessary to test for sialic acid for the drug product and drug substance in the follow-up stability program; however, Roche must continue to test and provide data on sialic acid for the ongoing registration batch stability program.

KEY AGREEMENTS ON CLINICAL DEVELOPMENT PROGRAM

During the development of MIRCERA®, FDA provided the following comments:

Pre-IND Meeting Feedback:

- Regarding Phase III trials, two adequate and well controlled trials for both maintenance and correction are required.

The phase III development program consisted of six pivotal trials: Two adequate and well-controlled studies in the correction setting and 4 adequate and well-controlled studies in the maintenance setting.

- If a substantial number of patients are utilizing Aranesp (darbepoetin alfa) at the time of MIRCERA® filing/approval, Roche must develop conversion techniques for these patients

Study BA17283 evaluates the proposed conversion techniques in order to maintain hemoglobin levels when converting from darbepoetin alfa.

End of Phase II Meeting Feedback

- FDA agreed in general with the proposed phase III study designs (patient population, endpoints, proposed dose, and statistical analysis plan). FDA advised that the clinical studies should have an adequate representation of minorities.

The phase II and phase III program combined consisted of 73% Caucasians, 20% Blacks (including African Americans), 5% Orientals and 9% Hispanics respectively.

- Regarding the maintenance studies, FDA recommended that Roche not switch patients from the comparator arm to MIRCERA to ensure that enough patients on the comparator arm for the same duration as MIRCERA to allow assessment of comparative safety and ultimate benefit-risk in the BLA submission.

In accordance with FDA's recommendation, the maintenance studies were designed to ensure that the patients on the comparator arm had the same duration of therapy as MIRCERA in order to allow comparative safety assessment.

- FDA agreed that the per-protocol analysis would be acceptable as the primary analysis for noninferioritiy approach. However, the data would also need to be analyzed for the intention to treat population.

The clinical study reports also provide the results analyzed using the Intent to Treat Population.

- FDA noted that since long term data on the use of MIRCERA will be important, Roche should consider rolling over patients who complete these studies into a long term uncontrolled extension study of MIRCERA.

A Phase IIIB roll-over study, BH18387 was established to collect long term safety information for up to 2 years from the phase II and phase III studies. As agreed with FDA at the Pre-BLA meeting, safety information (SAEs, Deaths, and Withdrawals) from this study would be provided at the time of filing and 4 month safety update.

Pre-BLA Meeting Feedback:

FDA provided the following feedback at the Pre-BLA meeting held on March 6, 2006:

- The results as presented in the Pre-BLA briefing package support a BLA filing for the treatment of anemia associated with CKD including patients on dialysis and not on dialysis. The results from the correction studies appear to support a BLA filing for a dosing recommendation of 0.6 mcg/kg IV or SC in patients not currently treated with an erythropoiesis stimulating agent. The results from the maintenance studies appear to support a BLA filing for the once every two weeks and the once every four week regimen when converting from epoetin or darbepoetin.

- FDA agreed that the safety data from the extension period of the two correction studies (i.e. data from 141 MIRCERA treated patients enrolled in the correction studies BA16736 and BA16738) be integrated and submitted at the time of the 4 month safety update as part of the routine regulatory process. However, as discussed at the Pre-BLA meeting, final listings for SAEs, deaths and withdrawals from the 141 RO0503821 treated patients and the 126 reference treated patients who had not completed the extension period at the time of the filing database closure will be included in the BLA.

- FDA agreed with the following approach for providing case report forms for the correction studies:

    - Case report forms will be provided for all deaths and withdrawals due to an adverse event at the time of the November 2005 database closure.

    - In addition, Case Report Forms will be provided for patients who had a serious adverse event associated with a study drug regimen alteration and patients who had a RBC transfusion that occurred in the correction/evaluation phase of the study and for those who completed the long term safety extension phase at the time of November 2005 database closure.

- FDA agreed with the approach of providing 2 datasets for the correction studies:

    - One dataset that encompasses all patient data for primary efficacy and safety evaluation from the correction/evaluation period (including 141 patients)

    - Another dataset that contains all patient data for patients who completed the extension period (not including the 141 patients) at the time of filing database closure

- FDA agreed that that the pooled safety results, proposed plans to address safety and the risk management plan support a BLA filing

Content/Format Agreements with FDA

FDA feedback was received on the content/format of the BLA on December 20, 2005 in response to a June 2005 submission outlining the content/format of the BLA. Additional feedback was also received at the Pre-BLA meeting.

Points to Consider	**Key Agreements with FDA**
BLA Submission Type	Agreement that the BLA be an electronic submission including the plan for the e-BLA folder structure and item table of contents
Narratives	Narratives for completed studies for Phase I, II and III. Narratives are provided for the following: 1) Subjects who withdraw from a study for any reason 2) Subjects who have study agent dose regimen alteration in association with serious adverse events 3) Subjects who receive red blood cell transfusions As agreed with Dr. Rieves on January 16, 2006, narratives for withdrawals due to kidney transplants would be waived
Application Summary, ISS and ISE	As requested by FDA, this BLA contains an Application summary, Integrated Summary of Safety and Integrated Summary of Efficacy
Case Report Forms	As agreed with FDA, the BLA contains case report forms for deaths, serious adverse events associated with a study drug regimen alteration, withdrawal due to an adverse event and subjects who have any red blood cell transfusion for completed Phase I, II and III studies. As agreed with FDA at the Pre-BLA meeting, for studies BA16736 and BA16738, Case Report Forms will be provided for all deaths and withdrawals due to an adverse event at the time of the November 2005 filing database closure. In addition, CRFs will be provided for patients who had a serious adverse event associated with a study drug regiment alteration and patients who had a RBC transfusion during the correction/evaluation phase of the study and for those who completed the extension period at the time of November 2005 filing database closure.

Points to Consider	Key Agreements with FDA
Datasets/Case Report Tabulations	As agreed with FDA in the content/format feedback as well as the Pre-BLA meeting, the following datasets would be provided: 1) <u>Datasets for Phase I Studies:</u> Studies: BP18034, BP18035, BP17278, BP16779 2) <u>Datasets for Phase II Studies:</u> For studies BA16285, BA16286, BA16528, 2 sets of datasets would be provided. One dataset contains the core treatment period. The second dataset contains the core period and the optional extension period combined. For study BA16260, only one dataset would be provided since this study did not contain an extension period. 3) <u>Datasets for Phase III Studies:</u> For studies BA16739, BA16740, BA17283 and BA17284, one dataset is provided. As agreed with FDA, two sets of datasets are provided for studies BA16736 and BA16738. One dataset contains the correction/evaluation period which is the basis of the primary efficacy results. The second dataset contains data for all patients who completed the study as of the November 2005 filing database closure.
Patient Profiles	Agreement from FDA to not submit subject listings (i.e. Patient Profiles)
Pooling Strategy	FDA agreement with the plan for not pooling the efficacy findings from the studies. FDA agreement that the pooling of Phase II and Phase III studies for safety appears reasonable.
SAS Programs	Agreement to provide programs for the phase II and phase III studies in order to allow the reviewer to follow the logic for the creation of the analysis datasets and the primary and secondary efficacy outputs generated for the analysis.

FDA Agreements on Safety Database

After the October 2, 2003 End of phase II meeting, Roche engaged in a number of discussions with FDA on the overall safety database (October 22, 2003, November 6, 2003, and November 10, 2003). Based on an agreement with FDA, Roche committed to providing long term safety data (1 year and 2 year) on patients in each dosing interval at the time of BLA filing as well ad demonstrating an adequate number of dosing cycles. These estimates were detailed in safety database tables submitted to BB-IND 10158 in amendments S-070 and S-072 and were accepted by FDA.

The FDA accepted safety database was further enlarged due to an increased recruitment in the phase III studies and more accurate estimations of drop out rates resulting in a substantial increase in the amount of safety data available at the time of BLA filing. This updated safety database was submitted to FDA on February 8, 2005 (S-153) and agreement was reached with FDA on March 24, 2005. In addition, the following agreements were made at the pre-BLA filing:

- The overall safety and efficacy results support a BLA filing
- Agreement with FDA that the safety data from the 141 MIRCERA treated patients from the extension period of the correction studies BA16736 and BA16738 is integrated and submitted at the 4 month safety update as part of the routine regulatory process

Attachment 2: Reviewer's Guide for BLA

INTRODUCTION AND GENERAL INFORMATION

This BLA for MIRCERA® is to support an indication for the treatment of anemia associated with chronic kidney disease including patients on dialysis and not on dialysis. It is important to note that through out the BLA the laboratory code of RO0503821 is used for the product except in the professional package insert and patient package insert in which the proposed trade name of MIRCERA® and the non-proprietary name of pegserepoetin alfa is used. This Guide will address various content and format aspects of the BLA on an Item by Item basis as a review aide.

Each report or publication referenced in this BLA has received a unique four-digit reference number which is used each time the document is referred to. All referenced reports considered important to the review of this BLA have been included.

OVERVIEW OF BLA ON AN ITEM BY ITEM BASIS

As agreed to with FDA, this BLA is organized according to the e-sub folder structure and the item table of contents (TOC) containing documents in Common Technical Document (CTD) format.

Item 1: Overall Table of Contents

The overall table of contents for this application is presented below:

Item	Documents	Paper Archive Copy Volume Number	Electronic Archive Copy Folder
Item 1	Administrative Forms	1	blamain
Item 2	Labeling	n/a	labeling
Item 3	Summary	n/a	summary
Item 4	Chemistry, Manufacturing and Controls (CMC)	n/a	cmc
Item 5	Nonclinical Pharmacology and Toxicology	n/a	pharmtox
Item 6	Human pharmacology and bioavailabilty/bioequivalence	n/a	hpbio
Item 7	Clinical Microbiology	n/a	n/a
Item 8	Clinical	n/a	clinstat
Item 9	Safety Update Report	n/a	n/a
Item 10	Statistical	n/a	stats
Item 11	Case Report Tabulations	n/a	crt
Item 12	Case Report Forms	n/a	crf
Item 13	Patent Information	1	other
Item 14	Patent Certification	n/a	n/a
Item 15	Establishment Description	n/a	other
Item 16	Debarment certification	n/a	other
Item 17	Field Copy certification	n/a	other
Item 18	Use Fee Cover Sheet	1	other
Item 19	Financial Information	1	other
Item 20	Other – Confidentiality Statement	n/a	other

Item 2: Labeling

Roche is providing the following in the labeling section of this BLA:

- Proposed Package Insert
- Proposed Patient Package Insert (one for vials and one for prefilled syringes)
- Container Labels for Vials
- Container Labels for Prefilled Syringes
- Unit Carton Labels (1 pack and 10 pack)
- Unit Carton Labels for Prefilled Syringes (1 pack and 10 pack)
- Blister pack for Prefilled syringes

It is important to note that a color coding system was developed for both the vials and prefilled syringes in order to facilitate the visual differentiation of the available strengths for post marketing use. A unique color will be utilized for the various strengths.

All documents related to labeling are provided in Item 2 and have the appropriate file names, bookmarks and hypertext linking. In addition, the labeling in SPL format is located in Item 2.

Item 3: Summary

Based on FDA's feedback, Roche is providing an Application Summary for this BLA. Please refer to section 4 of the Application Summary for an overview of the key agreements with FDA on the development program for MIRCERA®. The Application Summary also contains the annotated label which provides links to the supporting documents.

Item 4: CMC

Item 4 provides all Chemistry, Manufacturing and Controls (CMC) information regarding MIRCERA®, including the complete body of data for EPO starting material, drug substance, drug product vials and drug product pre-filled syringes. The documents contained in this E-sub structure follow the CTD format for each section as well as Appendices and Regional Information. Accordingly, the Quality Overall Summary is an overview of the scope and outline of the complete body of data for CMC (Module 3). Roche is also providing the following documents in the Regional Information section of this BLA:

- Executed batch records for one batch each of EPO starting material, drug substance, drug product vials and drug product pre-filled syringes
- Environmental Assessment (Categorical Exclusion Certification)
- Investigational Formulations/Batch Tracking for materials used to support this license application
- Comparability Program for Post Approval Changes
- Proposal for Exemption from Bulk Sterility Testing
- Transport Evaluation
- Description of the Roles and Responsibilities of each production site.

In accordance with the FDA "Guidance for Industry M4Q: The CTD-Quality" (August 2001), the methods validation for EPO starting material and drug substance may be found in the respective sections 3.2.S.4.3. For Drug Product Vials and Prefilled Syringes, methods validation may be found in the respective sections 3.2.P.5.3.

Item 5: Nonclinical (pharmtox)

Item 5 includes all preclinical information regarding MIRCERA®. The documents contained in this E-sub structure are CTD documents following the CTD TOC for each document. Accordingly, the Nonclinical Overview, and Nonclinical Written and Tabulated Summaries are provided within Item 5. The Nonclinical written and tabulated summaries fulfill the requirements of the pharmacology and toxicology summaries. In addition to the high level summaries (Nonclinical Overview, Nonclinical Written and Tabulated Summaries), this section includes 13 pharmacology studies, 4 analytical validation reports, 5 pharmacokinetic studies, 8 toxicology studies, 6 reproductive toxicology studies, 3 local tolerance studies. In addition, an in vitro cell proliferation study and a tissue binding study were conducted in lieu of formal carcinogenicity studies as agreed with FDA at the End-of-Phase II meeting.

The table below provides an overview of Item 5 of the BLA:

Studies	**Reference Numbers**
Summaries	Nonclinical Overview, Nonclinical Written and Tabulated Summaries
Pharmacology Studies	2000, 2001, 2002, 2003, 2004, 2005, 2006, 2007, 2008, 2010, 2011, 3011, 2009
Analytical Methods and Validation Reports	3000, 3001, 3002, 3003
Pharmacokinetic Studies	3006, 3007, 3008, 3009, 3010
Toxicology Studies	1000, 1001, 1002, 1003, 1004, 1005, 1006, 1007
Reproductive Toxicology Studies	1011, 1012, 1013, 1014, 1015, 1016
Local Tolerance Study	1017, 1018, 1019
Other Studies (in lieu of Carcinogenicity Studies)	1008, 1009

Item 6: Human Pharmacology

Item 6 includes clinical pharmacology information for MIRCERA®. The documents contained in this E-sub structure are CTD documents following the CTD TOC for each document. Accordingly, the Summary of Biopharmaceutic Studies and Associated Analytical Methods and the Summary of Clinical Pharmacology Studies documents are provided in Item 6. In addition, the Analytical Summary is provided in this item of the BLA. It is important to note that a few reports are legacy documents and therefore appear in the modular structure.

The following is an outline of the items provided in Item 6:

Studies	Protocol/Reference Numbers
Summaries	Summary of Biopharmaceutics and Associated Analytical Methods Summary of Clinical Pharmacology Studies Analytical Summary
Bioavailability (BA) Study Reports	BP16964/4000
Validation Reports for Pharmacokinetic Measurements	Study 1017795/4005, Study 1020238/4014, Study 1004990/4001, Study 1011014/4002, Study 1003539/4006, Study 1016902/4003, Study 1020758/4004
Validation for Antibody Measurements	Study 1020759/4012, Study 1020757/ 4013, Study 1012170/4007, Study 1016886/4010; Study 040324/4008, Study 040323/4009
Studies Using Other Human Biomaterials	Study 1019685/4011
Healthy Subject PK and Initial Tolerability Studies	BP16198/6000, BP16239/6005, JP16417/6007, JP16690/6004, BP16346/6001, WP16422/6003, WP16383/7000, BP18035/7004, BP17278/6006, BP17570/7001
Patient PK and Initial Tolerability Studies	BP16779/7002, BP18034/7003
Population PK Studies	Phase I Population PK/6012, Phase II Population PK/6008, Phase III Population PK/6009, Phase III Trial Simulations/6010

As agreed with FDA in the Content/Format submission (June 2005), datasets are provided for studies BP16779 (MRN 7002), BP18034 (MRN 7003), BP18035 (MRN 7004), and BP17278 (MRN 6006), In addition, datasets are provided for the phase II and phase III population pharmacokinetic studies (MRN 6008, 6009).

Item 6 also contains a trial simulation report (MRN 6010) as well as the datasets which further support the dose of 0.6 mcg/kg dose in the correction setting as well as the support to recommend a once monthly dosing schedule (instead of a once every four weeks) when converting from epoetin alfa or darbepoetin alfa.

Item 7: Clinical Microbiology

There will be no clinical microbiology section as this section is not applicable for this BLA.

Item 8: Clinical

The documents contained in this E-Sub structure are CTD documents following the CTD TOC for each document. Therefore, Item 8 contains the Clinical Overview, Summary of Biopharmaceutics Studies and Associated Analytical Methods, Summary of Clinical Pharmacology Studies. In accordance with FDA's request, an Integrated Summary of Safety and Integrated Summary of Efficacy are provided. In addition, an Integrated Summary of Benefits and Risks is also provided in Item 8.

Item 8 also provides the complete final clinical study reports for all Phase II and Phase III clinical studies in renal anemia to support this application. Datasets are also provided for each phase II and phase III study. It is important to note that 2 sets of datasets are provided for studies BA16736 and BA16738. The dataset BA16736 and BA16738 (Core Period) contains all patient data for the primary efficacy and safety evaluation from the correction/evaluation period. The dataset entitled BA16738 and BA16738 (Completers) includes all data for patients who completed the extension period at the time of the filing database closure.

There are a number of studies being conducted in Japan to support the Japanese development program. As agreed with FDA on the content/format of the BLA, study report synopses are being provided for these Japanese studies since this information is not considered pivotal for the determination of safety and efficacy. However, safety information from these studies (i.e. deaths, SAEs) are included in the Integrated Summary of Safety.

The Risk Management Plan is provided in Item 8. In addition, this Item includes the Synopsis of Individual Studies and a Tabular Listing of all clinical studies.

Studies	Reference Numbers
Summaries	Clinical Overview (9002) Summary of Biopharmaceutics and Associated Analytical Methods (9004) Summary of Clinical Pharmacology Studies (9017) Integrated Summary of Efficacy (9009) Integrated Summary of Safety (9008) Risk Management Plan (9016) Integrated Summary of Benefits and Risks (9034) Synopses of Individual Studies (9020) Tabular Listing of all Clinical Studies (9007) Compliance with PREA (9006)
Controlled Clinical Studies (Phase III Studies)	Studies BA16736 (8000), BA16738 (8001), BA16739 (8004), BA16740 (8005), BA17283 (8006), BA17284 (8007)
Uncontrolled Clinical Studies (Phase II Studies)	Studies BA16260 (8002), BA16528 (8003), BA16528 Extension (8010), BA16285 (8008), BA16285 Extension (8011), BA16286 (8009), BA16286 Extension (8012)
Other Studies	Study Report Synopses supporting Japanese Development Proram (9014)
Other Information	List of INDs, Compliance Statements, Abuse and Dependency

Item 9: Safety Update

The safety update report for this BLA will be submitted 4 months following this submission and if relevant, any updates to the draft labeling.

Item 10: Statistical

Item 10 includes SAS programs, macros and documentation for the following studies outlined below. Roche has provided SAS programs and SAS macro programs in ASCII text format using the .sas file extension. As previously agreed with FDA, the submitted programs are in their original setup for the Roche UNIX environment and therefore not executable in the FDA environment. The programs and the SAS macro programs allow the reviewer to follow the logic for the creating of the analysis datasets and the primary and secondary efficacy outputs for the generated analysis.

Studies	**Protocol Numbers**
Phase III Studies	BA16739, BA16740, BA17283, BA17284, BA16736, BA16738
Phase II Studies	BA16285, BA16286, BA16260, BA16528
ISS/ISE	Analysis of pooled data from Phase II and Phase III studies are provided.
Trial Simulation	Population PK/PD analyses of Studies BA16736, BA16739, BA16740

Item 11: Case Report Tabulations

As agreed with FDA, patient profiles are not provided since datasets are provided. Datasets and data definition files are provided for the following studies:

Studies	**Protocol Numbers**
Phase I Studies	BP17278, BP18034, BP18035, BP16779. For studies, BP18034, BP18035 and BP16779, the datasets are provided in both excel and sas xpt format for ease of FDA review.
Phase II Population Pharmacokinetics	Consists of studies BA16260 and BA16528
Phase III Population Pharmacokinetics	Consists of studies BA16736, BA16739, and BA16740
Trial Simulations	Population PK/PD analyses of Studies BA16736, BA16739, BA16740
Phase II Studies	For studies BA16285, BA16286, BA16528, 2 sets of datasets are provided. One dataset contains the core treatment period. This dataset should be used for the primary analysis. The second dataset contains the core period and the optional extension period combined. For study BA16260, only one dataset is provided since this study did not contain an extension period
Phase III Studies	For studies BA16739, BA16740, BA17283 and BA17284, one dataset is provided. As agreed with FDA, two sets of datasets are provided for studies BA16736 and BA16738. One dataset contains all patient data for the primary efficacy and safety evaluation from the correction/evaluation period. The second dataset contains all data for patients who completed the extension period at the time of the filing database closure.
Integrated Safety Dataset	Pooled Phase II/III Dataset

Item 12: Case Report Forms

As agreed with FDA in the content/format feedback (December 2005), the Sponsor is providing CRFs for deaths, dropouts, as well as subjects with: any serious adverse event associated with a study drug regimen alteration; withdrawal due to an adverse event; and subjects who received any red blood cell transfusions for all the following completed studies. Details are provided below:

Studies	**Protocol Numbers**
Phase I Studies	CRFs are provided for those patient who met the above criteria for the following phase I studies: WP16422, BP16346, WP16383, BP17278, BP17570, BP16964, BP16779 For studies BP16198, BP16239, JP16690, JP16417, BP18035, BP18034, no case report forms are provided since no patient met the above criteria that required submission of CRFs.
Phase II Studies	CRFs are provided for those patients who met the above criteria for the following studies: BA16285, BA16286, BA16260, BA16528
Phase III Studies	CRFs are provided for those patients who met the above criteria for all phase III studies: BA16739, BA16740, BA17283, BA17284 As agreed with FDA at the pre-BLA Meeting, for studies BA16736 and BA16738, Case Report Forms will be provided for all deaths and withdrawals due to an adverse event at the time of the November 2005 filing database closure. In addition, CRFs will be provided for patients who had a serious adverse event associated with a study drug regimen alteration and patients who had a RBC transfusion during the correction/evaluation phase of the study and for those who completed the extension period at the time of November 2005 filing database closure.

Items 13 & 14: Patent Information and Patent Certification

Only Item 13, Patent Information, is provided as Patent Certification is not applicable to this BLA.

Item 15: Establishment Description

Establishment description is provided.

Item 16: Debarment Certification

The original, signed Debarment Certification is provided in Item 16 of this BLA.

Item 17: Field Copy Certification

As agreed with FDA on March 30, 2006, a field certification letter is not being submitted to the FDA district office since the DMPQ/TFRB Group at FDA will review the CMC section of the BLA to facilitate the prior approval inspection and not the district office.

Item 18: User Fee

The User Fee payment for this original BLA was previously wired to FDA. The User Fee ID number is PD3004367. The user fee cover sheet (Form FDA 3397) is provided in Item 18 of this BLA.

Item 19: Financial Disclosure

Investigator financial disclosure information is provided in Item 19 of the BLA. Signed Form FDA 3454 with an attachment listing investigators who do not have financial information to disclose are provided in this Item.

Item 20: Confidentiality Statement

The Confidentiality statement is provided.

EXHIBIT F-2

GRASS System 03117. 12554

DEPARTMENT OF HEALTH & HUMAN SERVICES

Public Health Service

Food and Drug Administration
Rockville, MD 20852

N2006-01443

MAY 08 2006

Hoffman La-Roche
Attention: Krishnan Viswanadhan, Pharm.D.
Director, Regulatory Affairs
340 Kingsland Street
Nutley, NJ 07110

Dear Dr. Viswanadhan:

We have received your biologics license application (BLA) submitted under section 351 of the Public Health Service Act for the following biological product:

Our Submission Tracking Number (STN): BL 125164/0

Name of Biological Product: Pegserepoetin alfa

Indication: Treatment of anemia associated with chronic kidney disease, including patients on dialysis and patients not on dialysis

Date of Application: April 18, 2006

Date of Receipt: April 19, 2006

User Fee Goal Date: February 17, 2007

All applications for new active ingredients, new dosage forms, new indications, new routes of administration, and new dosing regimens are required to contain an assessment of the safety and effectiveness of the product in pediatric patients unless this requirement is waived or deferred. We reference the deferral granted on February 21, 2006, for the pediatric study requirement for this application.

We request that you submit all future correspondence, supporting data, or labeling relating to this application in triplicate, citing the above STN number. Please refer to http://www.fda.gov/cder/biologics/default.htm for important information regarding therapeutic biological products, including the addresses for submissions.

Effective August 29. 2005, the new address for all submissions to this application is:

Food and Drug Administration
Center for Drug Evaluation and Research
Therapeutic Biological Products Document Room
5901-B Ammendale Road
Beltsville, MD 20705-1266

We will notify you within 60 days of the receipt date if the application is sufficiently complete to permit a substantive review.

If you have any questions, please contact the Regulatory Project Manager, Florence O. Moore, M.S., at (301) 796-2050.

Sincerely,

Kyong "Kaye" Kang, Pharm.D.
Chief, Project Management Staff
Division of Medical Imaging and Hematology Products
Office of Oncology Drug Products
Center for Drug Evaluation and Research

MIRCERA®

TESTING PHASE - BB IND 10158

Communication	Date of Communication
Initial IND for the Treatment of Anemia Associated with Chronic Renal Failure	12/4/01
Telephone conference with FDA during which the FDA confirmed that the IND was effective as of 1/3/02	1/3/02
Response to FDA Telephone Request for Information (January 3, 2002)	1/10/02
CMC – Information Amendment	1/15/02
Information Amendment: Toxicology Report RR 1002693	2/01/02
Protocol Amendment: New Investigators Protocols BA16285 and BA16528	2/18/02
Other: Response to FDA Teleconference	2/18/02
Protocol Amendment: New Investigator Protocol BA16528	3/01/02
Protocol Amendment: New Protocol JP16690B	3/04/02
Information Amendment: CMC: Change in Ro 50-3821 Labels	3/05/02
Protocol Amendment: New Investigators Protocols BA16285, BA16286 and BA16528	3/15/02
Protocol Amendment: New Investigators Protocol BA16285	4/02/02
Protocol Amendment: Change in Protocol JP16690B to version D	4/05/02
Protocol Amendment: New Investigators Protocol BA16285 and BA16286	4/17/02
INFORMATION AMENDMENT: Chemistry, Manufacturing and Controls - Additional Drug Product Information and Stability Update	4/22/02
Protocol Amendment: New Investigator Protocol BA16285	4/26/02
General Correspondence: Update of Roche Contact Personnel	5/02/02
PROTOCOL AMENDMENT: Add Investigator Doc. Protocol BA16286 PROTOCOL AMENDMENT: New Investigators Protocol BA16528	5/03/02
PROTOCOL AMENDMENT: Additional Investigator Documentation for Protocols BA16285 and BA16286	5/06/02
PROTOCOL AMENDMENT: Change in Protocol BA16285C to version D	5/22/02
PROTOCOL AMENDMENT: Change in Protocol BA16528C to version D	5/22/02
PROTOCOL AMENDMENT: Change in Protocol BA16286C to version D	5/24/02
Response to FDA Request for Information (March 22, 2002)	5/30/02
PROTOCOL AMENDMENT: Change in Protocol BA16285D to version E	7/02/02
PROTOCOL AMENDMENT: Change in Protocol BA16286D to version E	7/02/02
PROTOCOL AMENDMENT: Change in Protocol BA16528D to version E	7/02/02
PROTOCOL AMENDMENT: New Investigators Protocols BA16285, BA16286 and BA16528 PROTOCOL AMENDMENT: Modification to Form FDA 1572 Previously Submitted	8/09/02

INFORMATION AMENDMENT: Revised Investigational Brochure	10/02/02
Information Amendment: Pharmacology/Toxicology: Final Study Report	10/09/02
INFORMATION AMENDMENT: Chemistry, Manufacturing and Controls – Additional Drug Product Information and Stability Update	10/10/02
INFORMATION AMEND: Clinical: Final Study Reports RR 1007694 and RR 1007693	10/15/02
PROTOCOL AMENDMENT: New Investigators Protocols BA16285 and BA16286	10/18/02
PROTOCOL AMENDMENT: Modification to Form FDA 1572 Previously Submitted	10/21/02
INFORMATION AMENDMENT: Chemistry, Manufacturing and Controls – Additional Drug Product Information and Stability Update	10/22/02
PROTOCOL AMENDMENT: New Investigators Protocols BA16285 and BA16528	11/05/02
PROTOCOL AMENDMENT: New Investigators Protocol BA16528	11/15/02
PROTOCOL AMENDMENT: New Investigators Protocol BA16286 and BA16528	12/09/02
INFORMATION AMENDMENT: Chemistry, Manufacturing and Controls – Changes in production of Drug Substance and formulation of Drug Product	12/13/02
PROTOCOL AMENDMENT: New Investigators Protocol BA16286	12/13/02
PROTOCOL AMENDMENT: New Investigators Protocols BA16285 and BA16286	12/20/02
PROTOCOL AMENDMENT: New Investigators Protocol BA16286	01/02/03

General Correspondence: Clinical Pharmacology Study Protocol	01/03/03
General Correspondence: Toxicology Study Protocols	01/17/03
Information Amendment: Pharmacology/Toxicology	02/14/03
IND Annual Progress Report	02/28/03
Protocol Amendment: New Protocol BP17278A	03/14/03
Information Amendment: Pharmacology/Toxicology	03/19/03
Information Amendment: Chemistry, Manufacturing and Controls – Additional Drug Product Information and Stability Update	03/27/03
General Correspondence: Update of Roche Contact Personnel	03/27/03
PROTOCOL AMENDMENT: New Investigators Protocol BA16286	04/04/03
PROTOCOL AMENDMENT: New Investigator Protocol BP17278	04/04/03
PROTOCOL AMENDMENT: New Investigators Protocol BA16528	04/23/03
PROTOCOL AMENDMENT: Change in Protocol - BA16285 to version F, BA16286 to version F, BA16528 to version F-G	05/13/03
General Correspondence: Request for Teleconference to Obtain FDA Guidance on Three General Issues for Clinical Development of Ro 50-3821 for Treatment of Chronic Renal Anemia	05/14/03
INFORMATION AMENDMENT: Comparability Protocol for planned drug substance process changes	05/16/03
PROTOCOL AMENDMENT: New Investigator Protocol BA16528 PROTOCOL AMENDMENT: Modification to Form FDA 1572 Previously Submitted	05/22/03
PROTOCOL AMENDMENT: Modifications to Form FDA 1572 Previously Submitted	6/04/03
PROTOCOL AMENDMENT: Modifications to Form FDA 1572 Previously Submitted for BA16528	6/25/03
PROTOCOL AMENDMENT: New Investigator Protocol BA16528	6/27/03
General Correspondence: Type B End of Phase II Meeting Request- RO0503821 for the Treatment of Chronic Renal Failure (Dialysis Program)	7/17/03

General Correspondence: Request for Type B End of Phase II Technical (CMC) Meeting	8/12/03
Information Amendment: Toxicology, Research Report 1009922	8/13/03
General Correspondence: Briefing Package to Support the September 18th End of Phase II Meeting	8/15/03
PROTOCOL AMENDMENT: Change in Protocol – BP17278 to version B	09/08/03
Response to Request for Information: Clarification of Safety Information and Technical Information for the Prefilled Syringe	09/12/03
General Correspondence: Briefing Package to Support the October 28th End of Phase II Meeting	09/26/03
INFORMATION AMENDMENT: Revised Investigational Brochure Version 6	10/01/03
PROTOCOL AMENDMENT: Change in Protocol – BP17278 to version C	10/09/03
GENERAL CORRESPONDENCE: Roche Minutes of October 2, 2003 End of Phase II Meeting	10/17/03
GENERAL CORRESPONDENCE: Updated Safety Database	10/20/03
Response to Request for Information: Clarification for End of Phase II Briefing Package	10/23/03
Response to FDA Request for Information: Roche Minutes of October 22, 2003 Follow-up Teleconference (Safety Database) and Updated Safety Database Table	10/27/03
Response to FDA Request for Information: Clarification of Safety Database for Patients Dosed at Every 4 Week Dosing Intervals	11/06/03
Response to FDA Request for Information: Proposed Protocol Text for "Loss of Effect" and Antibody Testing	11/11/03
Information Amendment: Chemistry, Manufacturing and Controls – Changes in Production of Drug Substance and Formulation of Drug Product	11/13/03
GENERAL CORRESPONDENCE: Roche Minutes of October 28, 2003 End of Phase II (CMC) Meeting	11/13/03
PROTOCOL AMENDMENT: New Investigator Protocol BA16286	11/20/03
PROTOCOL AMENDMENT: New Protocols (Phase III) BA16736, BA16739, BA16740 & BA17283	11/26/03
INFORMATION AMENDMENT: Chemistry, Manufacturing and Controls – Registration Plan for Drug Product Vials	12/09/03
PROTOCOL AMENDMENT: Protocols (Phase III) - BA16736B, BA16739, BA16740 & BA17283	12/19/03
INFORMATION AMENDMENT: Chemistry, Manufacturing and Controls – Additional Drug Product Information and Stability Update	12/22/03
Response to FDA Request for Information: Roche Minutes of November 10, 2003 Follow-up Teleconference (Safety Database)	01/13/04

Response to FDA Request for Information: Clarification of Roche Minutes of November 10, 2003 Follow-up Teleconference (Safety Database)	01/29/04
Chemistry, Manufacturing and Controls – Additional Drug Product Information and Stability Update	02/02/04
PROTOCOL AMENDMENT: New Phase III Protocol BA16738	02/02/04
IND Annual Progress Report	03/02/04

Chemistry, Manufacturing and Controls – Registration Plan for Drug Product Vials	03/04/04
PROTOCOL AMENDMENT: New Investigators for Protocols BA16736, BA16739 and BA16740	03/05/04
Response to FDA Request for Information: Additional copies of IND Annual Progress Report	03/05/04
INFORMATION AMENDMENT: Pharmacology/Toxicology – Research Reports 1009783 and 1010100	3/10/04
PROTOCOL AMENDMENT: New Investigators for Protocols BA16739 and BA16740	3/12/04
Response to Request for Information: Replacement Table 1 of Protocol BA16738	3/18/04
GENERAL CORRESPONDENCE: Roche Minutes of March 12, 2004 Teleconference to Discuss Statistical Considerations for Study BA16738	3/22/04
PROTOCOL AMENDMENT: New Investigators for Protocols BA16739 and BA16740	3/23/04
PROTOCOL AMENDMENT: New Phase I Protocols BP18034 and BP18035	3/26/04
PROTOCOL AMENDMENT: New Protocol (Phase III) – BA17284	3/31/04
PROTOCOL AMENDMENT: New Investigators for Protocols BA16736, BA16739 BA16740 and BA17283	4/05/04
PROTOCOL AMENDMENT: New Investigators for Protocols BA16736, BA16739 BA16740 and BA17283	4/13/04
PROTOCOL AMENDMENT: New Investigators for Protocols BA16736, BA16739 BA16740 and BA17283	4/21/04
INFORMATION AMENDMENT: Chemistry, Manufacturing and Controls – Introduction of a new dosage form (Prefilled Syringes) and Changes in Analytical Method for Puritity/Identity of Drug Product	4/30/04
PROTOCOL AMENDMENT: New Investigators for Protocols BA16736, BA16739 BA16740 and BA17283	5/04/04
PROTOCOL AMENDMENT: New Investigators for Protocols BA16739 BA16740 and BA17283	5/10/04
PROTOCOL AMENDMENT: New Investigators for Protocols BA16736, BA16739 BA16740 and BA17283	5/27/04
PROTOCOL AMENDMENT: New Investigators for Protocols BA16736, BA16739 BA16740 and BA17283	6/02/04
PROTOCOL AMENDMENT: New Investigators for Protocols BA16736, BA16739 BA16740 and BA17283	6/07/04
RESPONSE TO FDA REQUEST FOR INFORMATION: Protocol BA17284 – Statistical Analysis Plan to Assess Correlation of Covariates	6/11/04
PROTOCOL AMENDMENT: New Investigators for Protocols BA16285, BA16736, BA16738, BA16739, BA16740, BA17283 and BA17284	6/18/04
PROTOCOL AMENDMENT: Additional Investigator Information for Protocols BA16285 and BA16740	6/21/04
PROTOCOL AMENDMENT: New Investigators for Protocols BA16736, BA16738, BA16739, BA16740, BA17283, BA17284, BP18034 and BP18035	6/24/04
PROTOCOL AMENDMENT: New Investigators for Protocols BA16736, BA16738, BA16739, BA16740, BA17283 and BA17284	7/01/04
RESPONSE TO FDA REQUEST FOR INFORMATION: Protocol BA17284 – Results of Analysis to Assess Correlation of Covariates	7/06/04
PROTOCOL AMENDMENT: New Investigators for Protocols BA16736, BA16738, BA16739, BA16740, BA17283 and BA17284	7/13/04

PROTOCOL AMENDMENT: New Investigator for Protocol BA16739	7/16/04
PROTOCOL AMENDMENT: New Investigators for Protocols BA16736, BA16738, BA16739, BA17283 and BA17284	8/02/04
Response to Request for Information: Simulation Equations Information for Teleconference	8/10/04
PROTOCOL AMENDMENT: New Investigators for Protocols BA16736, BA16738, BA16739, BA17283 and BA17284	8/11/04
OTHER: Follow-up to the Phase III Protocols BA16738 and BA17284	8/12/04

RESPONSE TO FDA REQUEST FOR INFORMATION: Protocol BA17284 - Results of the Simulation Analysis to Assess Correlation of Covariates	8/16/04
PROTOCOL AMENDMENT: New Phase III Protocol BH18387	8/19/04
PROTOCOL AMENDMENT: New Investigators for Protocols BA16738, BA16739, BA17283 and BA17284	8/20/04
PROTOCOL AMENDMENT: New Investigators for Protocols BA16736, BA16738, BA16740 and BA17284	8/27/04
PROTOCOL AMENDMENT: New Investigators for Protocols BA16738, BA16740, BA17283 and BA17284	9/10/04
PROTOCOL AMENDMENT: New Investigators for Protocols BA16738 and BA17284	9/20/04
IND SAFETY REPORT – 7-Day Expedited Safety Report (MCN #380584) for Protocol BA16740	9/23/04
IND SAFETY REPORT: Initial Written Report and Analysis of Similar Events for MCN 380584	9/29/04
PROTOCOL AMENDMENT: New Investigators for Protocols BA16736, BA16738 and BA17284	10/7/04
INFORMATION AMENDMENT: Chemistry, Manufacturing and Controls – Registration Stability Plan for Drug Product Vials and Prefilled Syringes	10/13/04
PROTOCOL AMENDMENT: Change in Protocol BA16738A to Version B	10/14/04
IND SAFETY REPORT: 1st Follow-Up to a Written Report for MCN 380584	10/19/04
PROTOCOL AMENDMENT: New Investigators for Protocols BA16736, BA16738, BA16739 and BA17284	10/20/04
PROTOCOL AMENDMENT: New Investigator for Protocol BH18387	10/20/04
IND SAFETY REPORT: 2nd Follow-Up to a Written Report for MCN 380584	10/22/04
PROTOCOL AMENDMENT: New Investigators for Protocols BA16736, BA16738 and BA17284	11/03/04
PROTOCOL AMENDMENT: New Investigators for Protocols BA16738 , BA17284 and BH18387	11/18/04
IND SAFETY REPORT: 7-Day Expedited Safety Report (MCN 377520) for Protocol BA17283	11/30/04
IND SAFETY REPORT: 3rd Follow-Up to a Written Report for MCN 380584	12/1/04
INFORMATION AMENDMENT: Chemistry, Manufacturing and Controls – Changes in Analytical Methods and Specifications for Drug Substance	12/2/04
PROTOCOL AMENDMENT: New Investigators for Protocols BA16738 , BA17284 and BH18387	12/3/04
INFORMATION AMENDMENT: Chemistry, Manufacturing and Controls – Changes in Analytical Methods and Specifications for Drug Substance	12/8/04
INFORMATION AMENDMENT: Chemistry, Manufacturing and Controls – Registration Stability Plan for Drug Product Vials and Prefilled Syringes	12/8/04
IND SAFETY REPORT: Initial Written Report and Analysis of Similar Events for MCN 384835 from Protocol BA16739	12/15/04
INFORMATION AMENDMENT: Pharmacology/Toxicology	12/15/04
IND SAFETY REPORT: Follow-Up to a Written Report for MCN 377520	12/17/04
IND SAFETY REPORT: 1st Follow-Up to a Written Report for MCN 384835	12/21/04
IND Safety Report – 7-Day Expedited Safety Report (MCN 388138) for Protocol BA16740 Fax to I. Irony	12/22/04
IND Safety Report – 7-Day Expedited Safety Report (MCN 388138) for Protocol BA16740 Fax to F. Moore	12/22/04
Other: Modification to Serial Submission Numbers	1/03/05
IND Safety Report – 7-Day Expedited Safety Report (MCN 391047) for Protocol BA17283	1/04/05
IND SAFETY REPORT: Follow-up to a Written Report for MCN 388138	1/04/05
IND SAFETY REPORT: Follow-up to a Written Report for MCN 391047	1/05/05
PROTOCOL AMENDMENT: New Investigators for Protocols BA16738 , BA17284 and BH18387	1/06/05
IND SAFETY REPORT: 2nd Follow-Up to a Written Report for MCN 384835	1/06/05
PROTOCOL AMENDMENT: New Investigators for Protocols BA16738, BA16740, BA17284 and BH18387	1/27/05

PROTOCOL AMENDMENT: Change in Protocol BP18035A to Version B	2/2/05
OTHER: Request for FDA Comment - Updated Safety Database Table and Data Reporting and Analysis Manuals for Phase III Clinical Development Program	2/7/05
INFORMATION AMENDMENT: Clinical - Final Study Report 1006220	2/14/05
INFORMATION AMENDMENT: Chemistry, Manufacturing and Controls – Changes in Drug Substance Manufacturing Facility	2/18/05
IND Annual Report	3/2/05
PROTOCOL AMENDMENT: New Investigators for Protocols BA16738, BA16739, BA16740, and BH18387	3/9/05
PROTOCOL AMENDMENT: New Investigators for Protocol BA16736 and BH18387	4/8/05
PROTOCOL AMENDMENT: New Investigators for Protocol BH18387	4/27/05
PROTOCOL AMENDMENT: Change in Study Procedures and Administrative Manual (SPAM) for Protocol BA16736 to version 3	5/2/05
IND SAFETY REPORT: Initial Written Report and Analysis of Similar Events for MCN 376258 from Protocol BA16739	5/11/05
INFORMATION AMENDMENT: Pharmacology/Toxicology	5/12/05
I. PROTOCOL AMENDMENT: Modification to Previously Submitted Investigator II. PROTOCOL AMENDMENT: New Investigators for Protocols BA16738, BA17284 and BH18387	5/17/05
INFORMATION AMENDMENT: Chemistry, Manufacturing and Controls – Changes in Analytical Methods and Specifications for Drug Product Vials and Pre-filled Syringes	5/18/05
INFORMATION AMENDMENT: Chemistry, Manufacturing and Controls – Additional Comparability Information for Drug Substance Made in the Commercial Facility	5/26/05
PROTOCOL AMENDMENT: New Investigators for Protocol BH18387	6/1/05
IND Safety Report – 7-Day Expedited Safety Report (MCN 397603) for Protocol BA16739	6/2/05
PROTOCOL AMENDMENT: Change in Study Procedures and Administrative Manual (SPAM) for Protocol BA16740 to version 3	6/2/05
INFORMATION AMENDMENT: Clinical - Final Study Report 1017562	6/6/05
Cancellation of IND Safety Report – 7-Day Expedited Safety Report (MCN 397603) for Protocol BA16739	6/6/05
INFORMATION AMENDMENT: Clinical - Final Study Reports 1010925, 1013633 and 1014207	6/10/05
PROTOCOL AMENDMENT: New Investigators for Protocol BA17284 and BH18387	6/13/05
INFORMATION AMENDMENT: Clinical - Addendum to Investigator's Brochure	6/13/05
IND Safety Report – 7-Day Expedited Safety Report (MCN 374848) for Protocol BA17283	6/22/05
IND SAFETY REPORT: Initial Written Report and Analysis of Similar Events for MCN 374848 from Protocol BA17283	6/24/05
INFORMATION AMENDMENT: Clinical – Letter to Investigators of Studies BA17284, BA16736, BA16739, and BA16740	6/24/05
PROTOCOL AMENDMENT: New Investigators for Protocol BA17284 and BH18387	6/28/05
OTHER: Request for FDA Feedback - Content/Format Questions of Upcoming BLA and Data Reporting Analysis Manuals for High Level Summaries	6/29/05
INFORMATION AMENDMENT: Chemistry, Manufacturing and Controls – Additional Comparability Information for Drug Substance Made in the Commercial Facility	6/29/05
IND SAFETY REPORT: 1st Follow-Up to a Written Report for MCN 374848	7/06/05
IND SAFETY REPORT: 7-Day Expedited Safety Report (MCN 395594) for Protocol BA17284	7/18/05
PROTOCOL AMENDMENT: New Investigators for Protocols BA17284 and BH18387	7/19/05
IND SAFETY REPORT: Initial Written Report and Analysis of Similar Events for MCN 395594 from Protocol BA17284	7/20/05
PROTOCOL AMENDMENT: New Investigators for Protocol BH18387	8/16/05
IND SAFETY REPORT: 1st Follow-Up to a Written Report for MCN 376258 from Protocol BA16739	8/18/05
INFORMATION AMENDMENT: Chemistry, Manufacturing and Controls – Additional Comparability Information for Drug Substance Made in the Commercial Facility	8/19/05

IND SAFETY REPORT: Initial Written Report and Analysis of Similar Events for MCN 381478 from Protocol BA16739	8/23/05
PROTOCOL AMENDMENT: Change in Study Procedures and Administrative Manual (SPAM) for Protocol BA17284 to version 2	8/25/05
IND SAFETY REPORT: 1st Follow-Up to a Written Report for MCN 381478 from Protocol BA16739	8/31/05
Other: Type B Pre-BLA Technical (CMC) Meeting Request	9/7/05
INFORMATION AMENDMENT: Chemistry, Manufacturing and Controls – Additional Stability Information to Support Drug Substance Made in the Commercial Facility	9/9/05
PROTOCOL AMENDMENT: New Investigators for Protocols BA16736 and BH18387	9/12/05
IND SAFETY REPORT: 2nd Follow-Up to a Written Report for MCN 381478 from Protocol BA16739	9/26/05
INFORMATION AMENDMENT: Chemistry, Manufacturing and Controls – Meeting Minutes and Response to Teleconference of July 18, 2005	9/27/05
OTHER: Request for FDA Feedback – Response to FDA's Request for Clarification Content/Format Submission (S-174)	10/3/05
OTHER: Final Data Reporting and Analysis Manuals for Phase III Clinical Development Program	10/7/05
OTHER: REQUEST FDA FEEDBACK: Proposed Pediatric Development Plan, Request for Deferral and Waiver of Pediatric Assessment for RO0503821 in Chronic Kidney Disease	10/10/05
PROTOCOL AMENDMENT: New Investigators for Protocols BA16285 and BH18387	10/12/05
INFORMATION AMENDMENT: Clinical - Final Study Reports 1016695, 1018112 and 1018354	10/25/05
OTHER: Briefing Package for November 29, 2005 Pre-BLA Technical (CMC) Meeting	10/28/05
IND SAFETY REPORT: Initial Written Report and Analysis of Similar Events for MCN 421707 from Protocol JH18512	11/1/05
OTHER: Minutes of November 3, 2005 Teleconference and Follow-Up Information on Data to be Included in BLA and 4 Month Safety Update, and Request for Teleconference	11/17/05
IND SAFETY REPORT: 2nd Follow-Up to a Written Report for MCN 376258	11/18/05
IND SAFETY REPORT: 7-Day Expedited Safety Report (MCN 424721) for Protocol BH18387	11/21/05
IND SAFETY REPORT: Initial Written Report and Analysis of similar Events for MCN 424721 for Protocol BH18387	12/1/05
INFORMATION AMENDMENT: Revised Investigational Brochure Version 7	12/2/05
IND SAFETY REPORT: 7-Day Expedited Safety Report (MCN 426903) for Protocol BA16736	12/7/05
INFORMATION AMENDMENT: Chemistry, Manufacturing and Controls – Qualification of a Fermentation Media Component for Epoetin Beta Starting Material and Changes in an Analytical Method for Purity of Drug Substance	12/8/05
IND SAFETY REPORT: 7-Day Expedited Safety Report (MCN 426946) for Protocol BH18387	12/8/05
Cancellation of IND Safety Report – 7-Day Expedited Safety Report (MCN 426903) for Protocol BA16736	12/12/05
PROTOCOL AMENDMENT: New Investigators for Protocols BA16739 and BH18387	12/13/05
IND SAFETY REPORT: Initial Written Report and Analysis of Similar Events for MCN 426946 for Protocol BH18387	12/16/05
OTHER: Type B Clinical Pre-BLA Meeting Request	12/22/05
IND SAFETY REPORT: 1st Follow-Up to a Written Report for MCN 421707 from Protocol JH18512	1/10/06
PROTOCOL AMENDMENT: New Investigators for Protocols BA16286, BA17823 and BH18387	1/12/06
OTHER: Update of Roche Contact Personnel	1/13/06
IND SAFETY REPORT: 7-Day Expedited Safety Report (MCN 426903) for Protocol BA16736	1/17/06
IND SAFETY REPORT: Initial Written Report and Analysis of Similar Events for MCN 426903 for Protocol BA16736	1/27/06
PROTOCOL AMENDMENT: New Investigators for Protocols BA16739 and BH18387	2/1/06

OTHER: BRIEFING PACKAGE for March 6, 2006 Pre-BLA Meeting	2/3/06
GENERAL CORRESPONDENCE: Response to FDA Meeting Summary of December 21, 2005 Pre-BLA (CMC) Meeting	2/10/06
IND SAFETY REPORT: 7-Day Expedited Follow-up Safety Report (MCN 424721) for Protocol BH18387	2/20/06
IND SAFETY REPORT: 1st Follow-Up to a Written Report for MCN 426903 for Protocol BA16736	2/22/06
IND SAFETY REPORT: 1st Follow-Up to a Written Report for MCN 424721 for Protocol BH18387	2/24/06
INFORMATION AMENDMENT: Clinical - Final Study Reports 1010924 and 1011799	2/24/06
IND SAFETY REPORT: 7-Day Expedited Safety Report (MCN 437568) for Protocol ML19382	3/1/06
IND Annual Progress Report	3/3/06
INFORMATION AMENDMENT: Chemistry, Manufacturing and Controls – Qualification of a Fermentation Media Component for Epoetin Beta Starting Material and Changes in an Analytical Method for Purity of Drug Substance	3/9/06
IND SAFETY REPORT: Initial Written Report and Analysis of Similar Events for (MCN 437568) for Protocol ML19382	3/9/06
IND SAFETY REPORT: 1st Follow-Up to a Written Report for MCN 437568 for Protocol ML19382	3/17/06
INFORMATION AMENDMENT: Pharmacology/Toxicology Research Reports N-181656, 1004874, 1004870, 1012025, 1010329, 1014495, 1016434, 1017234, 1019698, 1019699, 1019700, 1019701, 1019702 and 1019704	3/24/06
INFORMATION AMENDMENT: Clinical - Final Study Reports 1020347 and 1019359	3/29/06
IND SAFETY REPORT: 2nd Follow-Up to a Written Report for MCN 437568 for Protocol ML19382	3/29/06
INFORMATION AMENDMENT: Clinical - Final Study Reports 1020388 and 1019351	3/31/06
General Correspondence: Clarification of Pre-BLA Meeting Minutes	3/31/06
IND SAFETY REPORT: 7-Day Follow-up Safety Report (MCN 426903) for Protocol BA16736	3/31/06
INFORMATION AMENDMENT: Clinical - Final Study Reports 1011051 and 1010921	4/5/06
CORRESPONDENCE: Trade Name Request	4/5/06
IND SAFETY REPORT: 2nd Follow-Up to a Written Report for MCN 426903 for Protocol BA16736	4/6/06
INFORMATION AMENDMENT: Clinical - Final Study Reports 1015889 and 1019351	4/14/06
INFORMATION AMENDMENT: Clinical - Final Study Reports 1018862	4/17/06
INFORMATION AMENDMENT: Clinical - Final Study Reports 1019467 and 1020062	4/18/06
INFORMATION AMENDMENT: Chemistry, Manufacturing and Controls	5/8/06
INFORMATION AMENDMENT: Pharmacology/Toxicology Research Reports 1020759, 1020757, 1012170, 1016886, BSL Report 040324 and BSL Report 040323	5/26/06
PROTOCOL AMENDMENT: New Investigators for Protocol BH18387	6/16/06
IND SAFETY REPORT: 7-Day Expedited Safety Report for MCN 454115 Protocol BH18387	7/12/06
PROTOCOL AMENDMENT: Change in Protocol BH18387A to Version B	7/13/06
IND SAFETY REPORT: 1st Follow-Up to a Written Report for MCN 454115 for Protocol BH18387 *(actually sent Initial Report)*	7/20/06
IND SAFETY REPORT: 1st Follow-Up to a Written Report for MCN 454115 for Protocol BH18387	7/28/06
IND SAFETY REPORT: 7-Day Expedited Safety Report for MCN 449565 Protocol BH18387	8/3/06

IND SAFETY REPORT: Initial Written Report and Analysis of Similar Events for MCN 449565 for Protocol BH18387	8/9/06
I. PROTOCOL AMENDMENT: Modification to Form FDA 1572 Previously Submitted II. PROTOCOL AMENDMENT: New Investigator for Protocol BA16738	8/24/06
PROTOCOL AMENDMENT: New Clinical Study Protocol ML20336	8/31/06

IND SAFETY REPORT: 7-Day Expedited Safety Report for MCN 461658 Protocol BH18387	9/15/06
CANCELLATION OF IND SAFETY REPORT: 7-Day Expedited Safety Report for MCN 461658 Protocol BH18387	9/19/06
IND SAFETY REPORT: 2nd Follow-Up to a Written Report for MCN 421707 from Protocol JH18512	9/22/06
OTHER: REQUEST FOR SPECIAL PROTOCOL ASSESSMENT (CLINICAL)	9/25/06
PROTOCOL AMENDMENT: Change in Protocol ML20336 to Version A	9/29/06
INFORMATION AMENDMENT: Revised Investigator's Brochure Version 8	10/3/06
GENERAL CORRESPONDENCE: Response to FDA Request for Information on the CREATE Study for NeoRecormon	10/18/06
PROTOCOL AMENDMENT: New Clinical Study Protocol BH20051	10/25/06
IND SAFETY REPORT: 3rd Follow-Up to a Written Report for MCN 437568 for Protocol ML19382 AE: died from an unknown cause	11/8/06
IND SAFETY REPORT: 7-Day Expedited Safety Report for MCN 467833 Protocol BH18387 AE: cognitive impairment, altered mental status, died unknown cause	11/9/06
CANCELLATION OF IND SAFETY REPORT: 7-Day Expedited Safety Report for MCN 467833 Protocol BH18387	11/13/06
IND SAFETY REPORT: 7-Day Expedited Safety Report for MCN 472242 Protocol ML19382 AE: Died	11/29/06
IND SAFETY REPORT: Initial Written Report and Analysis of Similar Events for MCN 472242 for Protocol ML19382 AE: died from an unknown causality	12/4/06
IND Annual Progress Report	12/15/06
IND SAFETY REPORT: 1st Follow-Up to an Initial Written Report for MCN 472242 for Protocol ML19382 AE: died from an unknown causality	12/18/06
INFORMATION AMENDMENT: Addendum to Investigator's Brochure Version 8	12/20/06
OTHER: REQUEST FOR FDA FEEDBACK: Proposed Pediatric Development Plan for RO0503821 in Chronic Kidney Disease (NH19707 and NH19708)	12/20/06
INFORMATION AMENDMENT: Chemistry, Manufacturing and Controls	12/20/06
IND SAFETY REPORT: 7-Day Expedited Safety Report for MCN 475699 Protocol NH19960 AE: Died	12/22/06
PROTOCOL AMENDMENT: New Clinical Study Protocol ML20338	12/22/06
PROTOCOL AMENDMENT: Change in Protocol ML20336 to Version B	12/22/06
IND SAFETY REPORT: 7-Day Expedited Safety Report for MCN 425194 Protocol BH18387	12/28/06
INFORMATION AMENDMENT: Pharmacology/Toxicology Report No. 1024499	1/4/07
IND SAFETY REPORT: Initial Written Report and Analysis of Similar Events for MCN 425194 for Protocol BH18387 AE: Death cause unknown	1/5/07
CANCELLATION OF IND SAFETY REPORT: 7-Day Expedited Safety Report for MCN 475699 Protocol NH19960 AE: Death Unexplained	1/5/07
IND SAFETY REPORT: 1st Follow-Up to an Initial Written Report for MCN 425194 for Protocol BH18387 AE: died due to myocardial infarction	1/17/07

IND SAFETY REPORT: 7-Day Expedited Safety Report for MCN 478043 Protocol NH19960 Conducted Under BB-IND 10964 AE: Death Unexplained	1/17/07

IND SAFETY REPORT: 7-Day Expedited Safety Report for MCN 477965 Protocol NH19960 Conducted Under BB-IND 10964 AE: Sudden Death	1/17/07
CANCELLATION OF IND SAFETY REPORT: 7-Day Expedited Safety Report for MCN 478043 Protocol NH19960 Conducted under BB-IND 10964 AE: Sudden Death	1/19/07
CANCELLATION OF IND SAFETY REPORT: 7-Day Expedited Safety Report for MCN 477967 Protocol NH19960 Conducted under BB-IND 10964 AE: Sudden Death	1/19/07
IND SAFETY REPORT: 7-Day Expedited Safety Report for MCN 440460 Protocol BH18387 Conducted Under BB-IND 10158 AE: Death Cause Unknown	1/24/07
IND SAFETY REPORT: Initial Written Report and 1st Follow-Up to an Initial Written Report and Analysis of Similar Events for MCN 440460 for Protocol BH18387 AE: Cerebrovascular Accident, Worsening of Cardiomyopathy and Death Cause Unknown	2/01/07
PROTOCOL AMENDMENT: Change in Protocol ML20338 to Version A	2/02/07
PROTOCOL AMENDMENT: New Clinical Study Protocol ML20337	2/06/07
INFORMATION AMENDMENT: Chemistry, Manufacturing and Controls	2/9/07
IND SAFETY REPORT: 2nd Follow-up to an Initial Written Safety Report, MCN 440460 AE: Cerebrovascular Accident, Worsening of Cardiomyopathy and Back Pain	3/06/07
PROTOCOL AMENDMENT: New Investigators for Protocols BH20051, ML20336 and ML20338	3/8/07
IND SAFETY REPORT: 7-Day Expedited Safety, MCN 486074 AE: Death Cause Unknown	3/12/07
IND SAFETY REPORT: Initial Written Safety Report, MCN 486074 AE: Death Cause Unknown Protocol: BH18387	3/20/07
IND SAFETY REPORT: 7-Day Expedited Safety Report, MCN 475699/2 AE: Died from a possible thromboembolism Protocol: NH19960	3/30/07
Cancellation of an IND SAFETY REPORT: 7-Day Expedited Safety Report, MCN 475699/2	4/02/07
IND SAFETY REPORT: 1st Follow-up to an Initial Written Safety Report, MCN 486074 AE: Death Cause Unknown Protocol: BH18387	4/12/07
PROTOCOL AMENDMENT: New Investigators for Protocols BH20051, ML20336 and ML20338	4/18/07
IND SAFETY REPORT: 7-Day Expedited Safety Report, MCN 493485 AE: Death Cause Unknown Protocol: BH18387	5/01/07
IND SAFETY REPORT: Initial Written Safety Report, MCN 493485 AE: Death Cause Unknown Protocol: BH18387	5/01/07
IND SAFETY REPORT: 1st Follow-up to an Initial Written Safety Report, MCN 493485 AE: Death Cause Unknown Protocol: BH18387	5/07/07
IND SAFETY REPORT: 7-Day Expedited Safety Report, MCN 495715 AE: Fatal Car Accident Protocol: JH20563	5/08/07
General Correspondence: Letter informing investigators of death imbalance in NH19960	5/08/07
PROTOCOL AMENDMENT: New Investigators for Protocols BH20051, ML20336 and ML20337	5/16/07
IND SAFETY REPORT: Initial Written Safety Report, MCN 495715 AE: Fatal Car Accident Protocol: JH20563	5/16/07

PROTOCOL AMENDMENT: New Investigators for Protocols BH20051, ML20336 and ML20338	5/22/07
IND SAFETY REPORT: 1st Follow-up to an Initial Written Safety Report, MCN 495715 AE: Fatal Car Accident Protocol: JH20563	5/22/07
IND SAFETY REPORT: 2nd Follow-up to an Initial Written Safety Report, MCN 495715 AE: Fatal suspected ventricular fibrillation Protocol: JH20563	6/04/07
PROTOCOL AMENDMENT: New Investigators for Protocols BH20051, ML20336, ML20337 and ML20338	6/25/07
IND SAFETY REPORT: 2nd Follow-up to an Initial Written Safety Report, MCN 472242 AE: Sudden Cardiac Death Protocol: ML19382	6/26/07
Other: Follow-up to FDA SPA Comments on NH20052, dated November 9, 2006 PROTOCOL AMENDMENT: New Protocol NH20052B	6/28/07
INFORMATION AMENDMENT: Pharmacology/Toxicology: Final Study Reports 1026439 and 1026440	7/02/07
PROTOCOL AMENDMENT: Change in Protocol BH18387B to Version BH18387B (US)	7/12/07
INFORMATION AMENDMENT: Pharmacology/Toxicology Publication Report 1025755	7/23/07
IND SAFETY REPORT: 7-Day Expedited Safety Report, MCN 503804 AE: Coma and fatal cerebral hemorrhage Protocol: JH20562	7/25/07
General Information: Response to FDA Letter of April 4, 2007 Regarding Erythropoiesis-stimulating agents (ESAs)	7/26/07
URGENT Request for FDA Feedback: Modification of Study Design for Study NH20052	7/27/07
PROTOCOL AMENDMENT: Change in Protocol BH20051A to Version C	7/27/07
OTHER: Draft Post Marketing Commitment Proposal and Request for Teleconference	7/27/07
IND SAFETY REPORT: Initial Written Safety Report, MCN 503804 AE: fatal cerebral haemorrhage and coma Protocol: JH20562	7/30/07
PROTOCOL AMENDMENT: New Investigators for Protocols ML20336, ML20337 and ML20338	8/06/07
IND SAFETY REPORT: 1st Follow-up to an Initial Written Safety Report, MCN 503804 AE: cerebral hemorrhage and coma Protocol: JH20562	8/20/07
IND SAFETY REPORT: 3rd Follow-up to an Initial Written Safety Report, MCN 495715 AE: Fatal suspected ventricular fibrillation Protocol: JH20563	8/20/07
IND SAFETY REPORT: 7-Day Expedited Safety Report, MCN 511554 AE: Death Cause Unknown Protocol: BH18387	8/20/07
IND SAFETY REPORT: 7-Day Expedited Safety Report, MCN 511605 AE: Death Cause Unknown Protocol: ML20336	8/20/07
IND SAFETY REPORT: 7-Day Expedited Safety Report, MCN 511763 AE: Death Cause Unknown Protocol: BH18387	8/20/07
INFORMATION AMENDMENT: Chemistry, Manufacturing and Controls – New Dosage Strengths for Pre-filled Syringes	8/23/07
IND SAFETY REPORT: Initial Written Safety Report, MCN 511554 AE: Death Cause Unknown Protocol: BH18387	8/27/07

IND SAFETY REPORT: Initial Written Safety Report, MCN 511605 AE: Death Cause Unknown Protocol: ML20336	8/27/07
IND SAFETY REPORT: Initial Written Safety Report, MCN 511763 AE: Death Cause Unknown Protocol: BH18387	8/27/07
IND SAFETY REPORT: 1st Follow-up to an Initial Written Safety Report, MCN 511554 AE: Death Cause Unknown Protocol: BH18387	9/06/07
IND SAFETY REPORT: 1st Follow-up Initial Written Safety Report, MCN 511605 AE: Death Protocol: ML20336	9/06/07
IND SAFETY REPORT: 2nd Follow-up Initial Written Safety Report, MCN 511605 AE: Ventriculation fibrillation Protocol: ML20336	9/11/07
PROTOCOL AMENDMENT: New Investigators for Protocols ML20336, ML20337 and ML20338	9/14/07
IND SAFETY REPORT: 2nd Follow-up to an Initial Written Safety Report, MCN 503804 AE: Cerebral hemorrhage and coma Protocol: JH20562	9/18/07
INFORMATION AMENDMENT: Revised Investigator's Brochure Version 9	9/20/07
PROTOCOL AMENDMENT: Change in Protocol NH20052B to Version C	9/20/07
IND SAFETY REPORT: 1st Follow-up to an Initial Written Safety Report, MCN 511763 AE: Cardiac arrest Protocol: BH18387	10/23/07
PROTOCOL AMENDMENT: New Investigators for Protocols BH20051, ML20336, ML20337 and ML20338	10/25/07
GENERAL CORRESPONDENCE: Letter to Investigators for IND Studies	10/30/07
INFORMATION AMENDMENT: Pharmacology/Toxicology Publication Report 1026974	11/16/07
PROTOCOL AMENDMENT: New Investigators for Protocols BH20051, ML20337 and NH20052	11/16/07
GENERAL CORRESPONDENCE: Letter to Investigators on the Termination of Studies ML20336, ML20337 and ML20338	11/21/07
IND SAFETY REPORT: 7-Day Expedited Safety Report, MCN 532100 AE: Death Cause Unknown Protocol: ML20511	11/21/07
INFORMATION AMENDMENT: Chemistry, Manufacturing and Controls – New Dosage Strengths for Pre-filled Syringes	11/28/07
IND Annual Progress Report	11/30/07

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APPLICATION PHASE - BL 125164/0

Correspondence	Date of Correspondence
Submission of BLA	4/18/06
Roche Action Plan for Cardiovascular Analysis (BLA) STN 125164/0	7/7/06
Chemistry Manufacturing and Controls – Pre-Approval Inspection (BLA) STN 125164/0	7/18/06
Clarification on 74-Day Letter - Preclinical Comments	8/11/06
4-Month Safety Update	8/14/06
FDA Requested Analyses on Safety in Relation to Absolute Hemoglobin and Rate of Rise of Hemoglobin	10/13/06
ECG Datasets	10/20/06
Chemistry, Manufacturing and Controls Information for Drug Product Vials and Pre-Filled Syringes ; Additional Stability Data, Process/Cleaning Validation and Warehousing Change (Item 4, Item 15)	10/27/06
Roche Action Plan for Submission of Additional Safety Information (BLA) STN 125164/0	11/3/06
Response to Request for Source Medical Documentation for Sudden Deaths	11/10/06
FDA Requested Preclinical Information	11/13/06
Response to Request for Information on Investigational Sites for BA16736 and BA16740	11/15/06
FDA Requested Information on C-Reactive Protein	11/28/06
Chemistry, Manufacturing and Controls Information for Drug Substance	11/30/06
FDA Request for Submission of ECG Data to ECG Warehouse	12/01/06
Additional Safety Information, Datasets, and Updated Labeling Supporting the BLA Review	12/4/06
FDA Request for Submission of Specific Site Information to Correlate Sites with Deaths	12/18/06
Chemistry, Manufacturing and Controls Information for Drug Substance	12/19/06
FDA Request for Clarification of Preclinical Toxicology Study	12/21/06
Request for Type A Meeting	1/3/07
FDA Request for Submission of Specific Site Information to Correlate Sites with Deaths	1/4/07
Response to FDA Request	2/2/07

Response to FDA Request for Information on CRP in Phase II Studies	2/6/07
Response to FDA Comments on Labeling	2/9/07
Response to Request for Information on Investigational Sites for CRTN 40380 and 40361	2/26/07
Response to FDA Request for Kaplan Meier Analyses	2/27/07
Response to FDA Request for Information on C-Reactive Protein	3/5/07
Chemistry, Manufacturing and Controls information for drug product vial and pre-filled syringes (Item 4)	3/22/07
Response to FDA Request for Revised Labeling	3/23/07
Response to FDA Request: Response to Questions 4 and 5 of March 20, 2007 FDA Fax	3/26/07
Chemistry, Manufacturing and Controls Information for Drug Product Vial and Pre-Filled Syringes	3/29/07
Response to FDA Request: Response to Questions 2, 3 and 6 of March 20, 2007 FDA Fax	3/30/07
Response to FDA Request: Response to Question 1 of March 20, 2007 FDA Fax	4/3/07
Chemistry, Manufacturing and Controls Information for Drug Substance	4/13/07
Response to FDA Request for Information on Creatinine Clearance Values in BP18034	5/3/07
Revised Draft Labeling and Response to FDA Feedback	5/7/07
Responses to FDA Request on Platelets and Bleeding Events	5/8/07
Revised Patient Package Insert and Instructions for Use	5/14/07
Package Insert in PLR Format	5/16/07
Chemistry, Manufacturing and Controls information—Responses to FDA Questions/Comments	5/17/07
General Correspondence: Summary of Teleconference with FDA	5/17/07
Response to FDA Request for Information	5/29/07
Summary of June 12, 2007 Teleconference and Request for Written Confirmation of Agreements	6/20/07
Chemistry, Manufacturing and Controls Information - DRAFT Responses to FDA Questions/Comments to Support the Teleconference Scheduled for July 27, 2007	7/18/07
Draft Response to Clinical and Non-Clinical Items from FDA Complete Response Letter issued May 18, 2007	7/31/07
Response to Complete Response Letter issued May 18, 2007 and Type A Meeting Request	8/13/07
Chemistry, Manufacturing and Controls information—Responses and Information to FDA Questions/Comments (Item 4) to Leachable and Extractable Study Reports	8/17/07
Amendment to Complete Response and Request for Class I Classification and Immediate Start of Review Clock	9/13/07
FDA Requested Analyses on Maximum Dosing and Transfusions	10/5/07
Response to FDA Comments on Carton and Container Labeling	10/9/07
Roche Comments on Draft Labeling for Mircera	10/15/07
Chemistry, Manufacturing and Controls information—Additional Clarification Information for Drug Substance and Drug Product Stability	10/16/07

Proposal for Post Marketing Commitments	10/16/07
Response to FDA Request: European Label for Mircera	10/17/07
Response to FDA Comments on Carton and Container Labeling (1000 mcg vials)	10/25/07
Roche Comments on Draft Labeling for Mircera	10/25/07
Revised Proposal for Post Marketing Commitments	10/30/07
Roche Comments on Draft Labeling for Mircera	11/6/07
Roche Comments on Draft Labeling for Mircera	11/9/07
Final Carton/Container Labeling for Mircera	11/14/07
OTHER: Product Correspondence - Final SPL for approved STN BL 125164/0	11/27/07